University of Alberta

Afferent regulation of phase transitions and muscle activity during walking in the cat.

by

Gordon William Hiebert ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Department of Physiology

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ABSTRACT

The act of locomotion is a result of the interaction of central neural mechanisms and afferent feedback from peripheral sensory receptors. One technique for studying how afferent feedback is used by the nervous system is to introduce a perturbation during walking and monitor how the animal responds to the challenge. This thesis examined the response of a walking cat to the sudden removal of ground support just prior to hindlimb foot contact. Upon encountering the hole in the supportive surface the limb is extended into the hole, then rapidly flexed out and replaced in front of the obstacle. At the same time there is an increase in contralateral knee extensor activity and a prolongation of stance phase. The observation that the corrective response to the absence of ground support occurred in chronic spinal cats, suggested that afferent feedback acting on the locomotor circuitry in the spinal cord was generating a very early initiation of swing phase activity. The introduction of group I afferent feedback from the extensor muscle spindles and tendon organs was demonstrated to be capable of suppressing and precisely regulating the timing of the onset of the corrective flexion reaction. Rapid stretch of the flexor muscle acting at the hip or ankle joint was shown to facilitate the onset of the flexion response out of the hole. It was further shown that during undisturbed locomotion afferent feedback indicating stretch of the flexor muscles was capable of promoting an early onset of the transition from stance to swing. These two observations indicate that afferent feedback signaling unloading and extension of the limb are involved in generating the initiation of the corrective flexion reaction and the timing of the normal stance-to-swing transition during walking. Analysis of the amplitude of extensor activity in the contralateral supporting limb, and the limb extended into the hole indicated that extensor group I afferents can potentially generate up to 80% of the extensor EMG activity during locomotion. In general, the afferent feedback was shown to effect the overall timing of the locomotor rhythm and to reinforce the amplitude of homonymous and functionally synergistic muscle groups.

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List of Abbreviations

LG	lateral gastrocnemius
MG	medial gastrocnemius
SOL	soleus
Pl	plantaris
VL	vastus lateralis
VM	vastus medialis
VI	vastus intermedius
IP	iliopsoas
ST	semitendinosus
TA	tibialis anterior
EDL	extensor digitorum longus
GTO	golgi tendon organ
EMG	electromyogram
ENG	electroneurogram
μm	micrometres
ms	millisecond
Hz	hertz, frequency
xT	intensity of nerve stimulation which generates a recorded potential at distal recording electrode
DSCT	dorsal spinocerebellar tract
VSCT	ventral spinocerebellar tract
DCL	dorsal column
DLF	dorso-lateral funiculus
CST	corticospinal tract

CHAPTER 1.

GENERAL INTRODUCTION

"The proprioceptive stimuli which are generated by the contraction of muscles taking part in the act play a regulative and not an intrinsic part in the act. Their chief importance may be in the grading of the individual component movements to the temporary exigencies of the environment."

T. Graham Brown, 1911

The neural control of movement is often thought of in terms of a hierarchical system. At the top of the hierarchy are the motor control regions of the cerebral cortex, which supposedly issue general commands such as "walk" or "stop". Next in the hierarchy are structures lying in the region of the brainstem. These structures take into account more immediate considerations, such as balance, and integrate them with the descending command before relaying them to the lowest level of the hierarchy. At the level of the spinal cord, the appropriate neuronal machinery is then activated to perform the desired task, while giving strong priority to information coming from the periphery. This system is analogous to that of the Boss telling the Foremen what to do, the Foremen then gathering the appropriate tools, and the Workers, who have knowledge of the state of affairs at the work site, then performing the task.

This thesis focuses on the lower level of this motor hierarchy. The main inquiry is how afferent feedback, primarily that of proprioceptive origin, regulates the spinal circuitry responsible for generating locomotion. Or more simply put; How does what is going on at the work site affect how the Workers perform their job? With this focus in mind, this Introduction is divided into three main sections. The first briefly reviews the literature demonstrating that the site of the basic neural circuitry responsible for generating locomotor activity is located in the spinal cord. The second outlines what is

known about the interaction of afferent feedback and locomotor circuitry, with emphasis on the role of muscle afferent feedback during walking in the cat. Finally, the basic objectives of the subsequent research chapters will be outlined.

1.1. Evidence for a Spinal Center for Locomotion.

By the beginning of this century evidence had been presented indicating that following acute transection of the spinal cord rhythmic alternation of the hindlimbs could be produced (Freusburg 1874, as cited by Wetzel and Stuart 1976). This observation indicated that the structures responsible for generating the basic locomotor rhythm were located in the lumbar region of the spinal cord. Thus, in the first part of the 20th century the site of the mechanisms involved were generally agreed upon and the constitution of this neuronal circuitry was the major focus of discussion.

In the early 1900s there were two schools of thought regarding the nervous control of locomotion. The first supported the idea that stepping was produced by the initiation of a chain of sequential reflexes. In this scenario the afferent signal generated by one movement would trigger a second, which would subsequently bring on a third and so on, eventually returning to the start of the cycle (Philippson 1905, as referenced in Wetzel and Stuart 1976; Sherrington 1910a,b). The second school of thought held that within the lower spinal cord there were centers capable of generating the alternation of the flexor and extensor muscles involved in locomotion. The most well known proponent of this line of thinking was T. Graham Brown. It was Brown's (1911) landmark demonstration that acutely spinalized and deafferented cats were capable of producing rhythmic stepping movements which "*show*[ed] *that the phasing of the acts of progression is determined neither by the peripheral skin stimuli nor by the self-generated proprioceptive stimuli of the muscles which take part in them*".

Brown described the alternating activation of flexor and extensor muscles as arising from the organization of the appropriate efferent neurons into mutually inhibitory antagonistic pairs. Activity in one half of the locomotor centre would prevent activity in the other half. As one "half centre" would fatigue and decrease in activity, the opposite centre would become active. While Brown postulated that the constituents of this circuit

were motoneurons, he was also aware of the possibility that interneurons acting between the half centres may be involved in the reciprocal inhibition. Further evidence of such a spinal organization came much later when it was demonstrated that administration of L-dopa to a paralyzed spinal cat opened a neuronal circuit with the properties implied by Brown's half-centre organization (Jankowska et al. 1967a,b). This circuit consisted of two groups of mutually inhibiting interneurons which had excitatory connections to either flexor or extensor motoneurons. The potential of this circuit to produce a locomotor rhythm has been well reviewed by Lundberg (1981).

Additional evidence that the basic neural circuitry underlying the generation of hindlimb locomotion resides in the lumbar region of the spinal cord has come from many other sources. Animals that have been spinalized and paralyzed, to prevent phasic afferent signals generated by movement of the limbs, have been shown to generate locomotor rhythms (Viala and Buser 1971; Perret 1973). Grillner and Zangger (1975, 1979) have essentially repeated Brown's earlier demonstration that a spinal and deafferented cat can produce rhythmic alternation of the hindlimbs.

1.2. Afferent Control of Locomotion.

While it is clear that the spinal cord is capable of generating the alternating extensor-flexor rhythm required for locomotion in isolation from peripheral afferent feedback or descending supraspinal input(Brown 1911; Grillner and Zangger 1979), it is also clear that peripheral input can shape the pattern of the muscle activity. Brown (1911) acknowledged that while afferent feedback does not cause the locomotor rhythm, such feedback is essential for regulating and adjusting the movements to accommodate for demands imposed by the environment. Many of the present day postulates developed to explain how afferent feedback regulates locomotor activity are extensions of the original concepts proposed by Sherrington (1910a,b) in his description of reflex stepping (ie. hip extension, cutaneous afferents from the paw, etc).

Perhaps the most dramatic demonstration of the power of afferent feedback in regulating locomotor activity is that of a spinal cat walking on a moving treadmill. In this situation, changes in the speed of the treadmill belt are immediately compensated for in a

manner similar to that seen in intact animals. An increase in the speed of the treadmill belt results in a decreased stance phase duration, while the duration of swing phase activity changes only slightly (Goslow et al. 1973; Grillner 1973; Forssberg et al. 1980; Halbertsma 1983). The fact that stance, rather than swing duration, is modified has led to the conclusion that afferent feedback generated during stance phase has a regulatory effect on the timing of extensor activity.

The timing of the locomotor cycle is not the only parameter that can be adjusted by afferent feedback. For locomotion to be truly adaptable, there must also be a means of regulating the intensity, or amplitude, of muscle contraction in order to compensate for sudden changes in the load. Sherrington (1910a) observed in the spinal cat that pressure to the foot pad resulted in a strong extension of the limb. More recently, it has been shown that resisting the extension movement of a limb in a decerebrate walking cat results in increased muscle activity, presumably to maintain a constant rate of contraction despite the increased load (Pearson and Duysens 1976; Duysens and Pearson 1980).

Much of the data presented in subsequent chapters of this thesis support the view that, it is predominantly the extensor activity associated with stance phase that is regulated by afferent feedback, either in terms of duration or amplitude of activity. However, when discussing the duration of stance phase activity, it must be kept in mind that one is inevitably also drawing conclusions in regard to factors influencing the timing of the onset of swing phase activity. Therefore, afferent regulation of the duration of extensor activity also implies that the stance-to-swing transition is being affected. In terms of how EMG amplitude is affected by afferent feedback, it is again the extensor muscles which have received much of the focus.

There are currently two dominant hypotheses regarding the afferent regulation of extensor activity during locomotion. One of these hypotheses dates back to the early observations of Sherrington (1910a), who reported that spinal stepping in dogs was most easily elicited when the animal was held so that the hip joint was extended. Grillner and Rossignol (1978) later demonstrated that the onset of swing phase activity in the hindlimb of a spinal cat could be prevented if the limb was not allowed to extend. Only when the hip joint was extended to a position similar to that normally occurring at the

end of stance phase was swing initiated and stepping resumed. Furthermore, other studies have demonstrated that sinusoidal flexion and extension of the hip joint can entrain locomotor activity in fictive spinal cats with the onset of flexor activity corresponding to the end of the extension movement (Anderson and Grillner 1983). These observations have led to the proposal that there is a position-dependant afferent signal generated by extension of the hip which acts to terminate extensor activity and initiate flexion.

The second hypothesis regarding the afferent regulation of extensor activity has its basis in studies performed on the cockroach. In 1973, Pearson and colleagues reported that activity in a force sensitive receptor, the campaniform sensilla, during limb retraction (stance phase) facilitated the activation of depressor motoneurons and inhibited levators (which are active during protraction, or swing phase). The afferent discharge of this receptor increases in response to loading of the limb, and decreases near the end of limb retraction as the load is passed to the other legs. This system seemed ideal to ensure that the initiation of protraction (swing) does not occur until there is sufficient unloading of the limb. These observations have subsequently been extended to walking in the cat. By clamping the ankle of a decerebrate walking cat in a flexed position, maintaining a load on the ankle extensor muscles, the onset of flexor activity could be prevented (Duysens and Pearson 1980). Only when the load was reduced to below a critical level would flexion of the limb resume. This led to the proposal that there must be a sufficient decrease in the afferent volley signalling loading of the limb before swing phase is initiated.

As will be discussed, there are a number of potential afferent sources signalling position of the limb or the amount of load carried by the limb. The subsequent sections review what is known about muscle, cutaneous, and joint afferents and how the reflex effects of these signals might explain the above postulates.

1.2.1. Muscle afferents

The research chapters presented in this thesis focus on the effects elicited by activation of large diameter muscle afferents, which include the group Ia muscle spindle afferent and the group Ib tendon organ afferent. Given this focus, the following two

sections give a short review of the properties of these proprioceptive receptors before discussing their potential role in regulating locomotor activity.

Golgi Tendon Organ

The Golgi tendon organs (GTO) are mechanoreceptors innervated by group Ib afferents, and are located between a small number (< 25) of muscle fibres and the tendinous points of origin or insertion. This location makes the GTO an excellent detector of changes in the tension produced between the muscle fibre and the tendon. GTO are highly sensitive to changes in tension produced by active contraction of muscle fibres that lie "in-series" with the receptor capsule (Houk and Henneman 1967), whereas force produced by passive stretch of the muscle is relatively ineffective in eliciting a response in the Ib afferents (Stephens et al. 1975) .

The fact that muscle contraction is the adequate stimulus for tendon organs, has raised the speculation that individual GTO discharge pattern should mirror the force profile produced by muscle activation. With one exception (Crago et al. 1982), this prediction has not held true. Most experiments, where a GTO is activated by electrically stimulating ventral root filaments in order to produce unfused contractions, have demonstrated that individual GTO activity does not consistently follow the force profile. A given tendon organ can respond with a greater discharge when activated by contraction of a weak slow motor unit than when activated by a stronger (greater force producing) fast motor unit (Gregory and Proske 1979). Often the discharge rate of a GTO is driven at the frequency of activity of an in-series motor unit regardless of the amount of force produced by the contraction. Furthermore, during asynchronous activation of 2 motor units, producing a smoother force profile, the GTO activity is driven at the stimulation rate of one or the other motor units (reviewed in Jami 1992). While individual GTO activity patterns may not provide an accurate indication of changes in force, it has been suggested that the ensemble discharge of GTO may provide a better indication. Experiments have shown that the average discharge frequency of a group of GTO does reflect dynamic changes in force and provide a slightly accurate indication of the overall force produced by the contraction (Horcholle-Bossavit et al. 1990).

Recordings of group Ib afferent activity during naturally occurring muscle activity have been rare. During naturally evoked muscle contractions (ie. crossed extensor reflex or stretch reflex) progressive increases in force result in step-like increases in tendon organ afferent discharge (Crago et al. 1982; Appenteng and Prochazka 1984). This phenomenon has been attributed to the recruitment of additional in series motor units during the muscle contraction. Interestingly, these step-like increases in the discharge frequency of the GTO do not appear to occur when multiple motor units are artificially activated (see above section). Furthermore, during stance phase of locomotion the discharge rate of group Ib afferents from extensor muscles do not display recruitment steps, rather single afferents tend to fire at a set frequency for the duration of the muscle activity (Loeb 1981; Appenteng and Prochazka 1984).

In many standard textbooks, the group Ib afferents from GTO have been described to produce disynaptic and trisynaptic inhibition of homonymous and synergistic motoneurons, while exciting motoneurons that are antagonistic to the receptor-bearing muscle. This reflex pathway has received a large amount of focus which has revealed that multiple systems (ie. cutaneous, muscle spindle and descending supraspinal inputs) converge onto the interneurons involved (reviewed in Jami 1992 and Jankowska 1992). However, it has recently been demonstrated that the "inverse myotactic reflex" (Laporte and Lloyd 1952), or "group I non-reciprocal inhibition" (Jankowska 1992) produced by tendon organ input, switches upon the onset of locomotion to excitation of synergistic motoneurons (Pearson and Collins 1993; Gossard et al. 1994).

The first evidence that GTO afferents from extensor muscles generate positive feedback onto synergists came from the observation that during locomotor activity isometric contraction of the triceps surae, induced by ventral root stimulation, could prolong extensor muscle activity and suppress flexor activity (Duysens and Pearson 1980). Conway et al. (1987) later demonstrated that during fictive locomotion in the cat, stimulation of extensor group I afferents could prolong extensor activation or act to reset the rhythm to extensor activity if introduced during the flexor phase. Once again, feedback from the GTO was implicated as the resetting effect could not be generated by

vibration of the muscle (a stimulus which selectively activates muscle spindle group Ia afferents), whereas ventral root stimulation producing muscle contraction (the adequate stimulus for GTO) was effective (see Fig. 7, Conway et al. 1987). During the course of this thesis work, conclusive evidence was obtained indicating that the group Ib afferents undergo a state dependent reversal in reflex action when the animal changes from a passive non-locomoting state to an active locomoting state (Pearson and Collins 1993; Gossard et al. 1994). Upon the onset of locomotion two distinct pathways have been shown to open. Gossard et al. (1994) demonstrated the existence of an oligosynaptic pathway from the group I afferents which depolarized extensor motoneurons. Furthermore, McCrea et al. (1995) demonstrated that a disynaptic excitatory pathway from extensor group I afferents onto extensor motoneurons opened upon locomotor activity, concurrent with a decrease in effectiveness of group I non-reciprocal inhibition.

Muscle Spindle

The muscle spindle typically consists of a bundle of 6 or more intrafusal muscle fibres situated "in-parallel" with the extrafusal muscle fibres. Thus contraction or stretch of the extrafusal muscle fibres will produce a change in the length of the muscle spindle. There are two types of afferent fibres innervating the muscle spindle, referred to as primary group Ia and secondary group II afferents. In general, both of these afferents exhibit a sensitivity to static changes in length, with the group Ia having an additional component of a greater dynamic sensitivity to the velocity of the length change (Cooper 1961). Together the afferents from the muscle spindle provide information regarding the length of the muscle and an indication of the rate at which a length change occurs.

The view of the muscle spindle becomes more complex by the fact that the sensitivity of spindle activity is controlled by gamma motoneuron innervation (Leksell 1945; Kuffler et al. 1951). There are two types of gamma innervation, commonly referred to as dynamic (γ_d) and static (γ_s) (Mathews 1962). In general, γ_d activity acts to increase the sensitivity of the group Ia afferent to the length change, whereas γ_s reduces the sensitivity of the group Ia, while increasing the overall level of discharge of both group Ia and group II afferents [for a more complete review of gamma innervation and

muscle spindle discharge see Gladden (1992)]. Moreover, the pattern of γ activity is regulated during walking. In the decerebrate walking cat it has been demonstrated that γ_d to muscle spindles in hindlimb extensors is phasically active, whereas γ_s is tonically active (Murphy et al. 1984; Taylor et al. 1985).

As a result of γ activity, the afferent discharge from the muscle spindles is not a simple reflection of the host muscle length. Without γ drive the extensor muscle spindle afferents would fire predominantly during swing phase, as the extensor muscles are stretched, and be relatively silent during stance when the muscle actively shortens. In general, the activity of most extensor muscle spindles have a biphasic pattern of activity during a normal step cycle (Prochazka et al. 1989). One peak of activity occurs during flexion of the limb and the second occurs during stance phase concomitant with contraction of the host muscle. The activity observed during stance is primarily a result of γ motoneurons activating the muscle spindles (Severin 1970, Loeb and Hoffer 1985). This efferent control of spindle activity has a number of implications when considering the functional role of muscle spindle afferents during locomotion.

The activation of group Ia muscle spindle afferents elicits monosynaptic excitation of homonymous motoneurons. The transmission of the monosynaptic reflex to extensor motoneurons is highly regulated during locomotion. The reflex is largest during stance, whereas during swing phase the group Ia input is relatively ineffective in producing extensor EMG activity (Akazawa et al. 1982). Much of this modulation in reflex amplitude has been demonstrated to occur as a result of the normal rhythmic changes in extensor motoneuron excitability (Shefchyck et al. 1984). Furthermore, presynaptic inhibition of the afferent transmission contributes to the phase-dependent regulation of the reflex (Stein and Capaday 1988).

Recently, an excitatory group Ia disynaptic pathway onto extensor motoneurons has been described during fictive locomotion in the cat (McCrea et al. 1995). Like that of the monosynaptic pathway, transmission through the disynaptic pathway is highly modulated in a phase dependent manner (Angel et al. 1996). There is also new evidence indicating that group Ia afferents can affect locomotion through a locomotor dependent oligosynaptic pathway (Guertin et al. 1995). Unlike the earlier results of Conway et al.

(1987), Guertin and colleagues demonstrated that during locomotion feedback from the group Ia afferents is capable of prolonging the duration of extensor activity and resetting the locomotor rhythm.

Potential role of muscle afferents in regulating locomotor activity.

At the time this thesis was started, there was a considerable amount of evidence indicating that proprioceptive afferent feedback from extensor muscles had the potential to regulate the duration and amplitude of muscle activity during locomotion. Much of the focus, at the time, was on the role of the extensor group Ib afferents. The observations of Duysens and Pearson (1980), Conway et al. (1987) and subsequently Pearson et al. (1992) indicated that the long-standing view of GTO feedback being inhibitory to homonymous muscle activity did not apply during locomotion. The fact that rhythmic activation of group Ib afferents could entrain or reset the locomotor rhythm suggested that these afferents were acting on the spinal circuitry generating the extensor/flexor pattern (Conway et al. 1987; Pearson et al. 1992). These early results fortified the postulate of Duysens and Pearson (1980) that GTO feedback during locomotion contributed to the maintenance of stance phase activity and the prevention of the onset of swing phase for as long as the limb was bearing weight.

Thus, one proposed role of group Ib feedback was to regulate the timing of the locomotor rhythm. In general, GTO feedback was thought primarily to ensure that swing did not occur until the limb was sufficiently unloaded. Later evidence demonstrated that not only is GTO feedback implicated in regulating the timing of stance phase activity, but could also contribute to the amplitude of extensor EMG generated during walking (Pearson and Collins 1993).

Until the results of Pearson and Collins (1993), facilitation of extensor EMG activity by proprioceptive feedback had mainly been attributed to muscle spindle group Ia afferents. The afferent volley produced by the slight yielding of the extensor muscles just after ground contact and that generated by gamma activation of muscle spindles has been proposed to generate a significant amount of muscle activity during walking. Severin (1970) demonstrated by preventing gamma motoneuron conduction to ankle

extensor muscles in the walking cat that the gamma induced spindle activity serves to produce approximately 50% of the muscle activity observed. The phasic modulation of monosynaptic reflex transmission supports the proposal that muscle spindle feedback reinforces extensor EMG activity. During swing phase it is beneficial that reflex transmission from extensor group Ia afferents is suppressed, otherwise reflex activation of the extensors could impede the flexion movement. Whereas during stance phase, the high level of reflex transmission would support the ongoing movement. The very recent evidence from McCrea and colleagues indicating the existence of excitatory disynaptic and oligosynaptic pathways, that open upon the onset of locomotion, provide further indication that the group Ia afferents are important for reinforcing extensor EMG activity (McCrea et al. 1995; Guertin et al. 1995; Angel et al. 1996).

So far much of this review of the potential role of muscle group I afferents during locomotion has emphasized their ability to prolong and facilitate extensor activity. However, there is still the issue of an afferent signal indicating limb position which terminates extensor activity and initiates flexion. The exact proprioceptive signal which indicates joint position is still a matter of contention. As will become evident in a subsequent section, joint receptor afferents might be capable of indicating limb position, although there is some debate on this issue. There is also the possibility that muscle spindle activity provides a sufficient indication of muscle length and thus a representation of the degree of limb extension. In general there is a decline in muscle spindle activity as the ankle and knee extensor muscles shorten during extension (Prochazka et al. 1989). Correspondingly, there is an increase in flexor muscle spindle activity as the pretibial flexors are stretched. It is not yet known whether a similar pattern of activity occurs for the primary flexors and extensors of the hip joint. If a similar pattern of activity does exist, this feedback would provide an ideal signal to indicate the position of the hip joint and generate the initiation of swing phase activity (Sherrington 1910a; Grillner and Rossignol 1978).

1.2.2. Cutaneous afferents

It has long been recognized that cutaneous feedback may have an important role in regulating limb activity during locomotion (ie. extensor thrust reflex - Sherrington 1910a). In the 1970's a phenomenon was documented which strongly emphasized that cutaneous reflexes adapt the locomotor rhythm to perturbations during walking. It was observed that if the dorsum of the paw or the anterior surface of the shank was touched during walking in the cat, the reflex response was different depending on whether the stimuli occurred during stance phase or during swing. Tactile stimulation of the foot dorsum during flexion resulted in an increase in flexor activity, while the same stimulus during stance generated an increase in extensor activity (Forssberg et al. 1975, 1977). The fact that this "phase-dependent reflex reversal" was observed in walking chronic spinal cats indicated that the reflex modulation was probably produced by the spinal circuitry responsible for generating the locomotor rhythm. Since the initial experiments of Forssberg and colleagues, a similar reversal of cutaneous reflexes has been shown to occur in intact quadrupedal walking animals (Forssberg 1979; Prochazka et al. 1978), as well as in humans (Duysens et al. 1990; Yang and Stein 1990).

This pattern of cutaneous reflex reversal dependant on the timing of the input makes the response functionally relevant: increased flexor activity during swing would bring the limb over an impeding obstacle, increased extensor activity during stance would resist potential displacement of the limb. However, the question still remains whether cutaneous feedback has a functional role during normal undisturbed locomotion. Unfortunately, the amount of data regarding the discharge pattern of cutaneous afferents from the dorsum of the foot during normal walking is fairly limited. It is possible that activation of these afferents by skin movement during locomotion, could reinforce the locomotor activity during the appropriate phases.

The more logical group of cutaneous afferents to investigate, if examining the afferent reinforcement of the undisturbed locomotor rhythm, would be those located on the plantar surface of the foot. Loeb (1981) reported that cutaneous mechanoreceptors located on the plantar surface (pad) of the foot normally discharge during stance phase. As alluded to earlier, Sherrington (1910a) demonstrated that pressure to the foot pad

resulted in a strong "extensor thrust reflex". This feedback may be utilized to reinforce the generation of stance phase activity.

Stimulation of the distal tibial nerve (a mixed nerve containing sensory afferents from the foot pad as well as motor nerves to the small muscles of the foot) during stance phase prolongs extensor activity (Duysens and Pearson 1976; Duysens 1977; however, Duysens and Stein 1978). Similar stimulation of the foot pad during swing phase, when these afferents are normally inactive, was shown to facilitate flexor activity. Thus, it appears that afferent feedback from the foot pad elicits the same reaction as afferents from the dorsum of the foot. However, recently Conway et al. (1994) and Guertin et al. (1995) have reported results somewhat contradictory to the earlier studies of Duysens and colleagues. Guertin and colleagues observed in fictive locomoting cats that distal tibial nerve stimulation during flexor activity resets the rhythm to extension. These different results may be explained by evidence that the distal tibial nerve appears to be functionally and anatomically segregated. Conway et al. (1994) demonstrated that after eliciting a late flexor reflex in a fictive cat treated with L-dopa [administration of L-dopa opens long latency reflex pathways which are presumed to reflect the organization of the spinal circuitry responsible for generating locomotion, see Jankowska et al. 1967a,b and Lundberg 1981)] stimulation of the lateral portion of the distal tibial nerve abruptly terminated the flexor activity, whereas the medial portion facilitated the existing flexor burst. It is apparent from these results that when assessing the reflex function of the cutaneous afferents one must be very careful to take into consideration the regions innervated by the afferents and whether afferents are normally active during a particular phase.

In general, there is very little evidence to support a postulate that cutaneous afferent input serves to regulate the timing of the normal locomotor rhythm. Upon denervation of the foot Sherrington (1910) observed no change in the overall locomotor rhythm. However, Duysens and Pearson (1976) have *postulated "that different afferent systems serve the same purpose and the proprioceptors of the limb* [following cutaneous denervation] *may take over the role jointly played be exteroreceptors and proprioceptors*". Whether phasically occurring afferent feedback from the foot pad acts

to regulate the locomotor rhythm remains a matter of conjecture. It is conceivable that as the limb is extended during stance the reduction of pressure on the foot pad may cause a drop in cutaneous feedback and that this decline in afferent feedback could signal the appropriate timing of swing phase onset, much like that postulated to occur from GTO feedback.

1.2.3. Joint Afferents

The potential role of joint afferents in the control of locomotion was brought to the forefront of attention by a series of experiments performed by Grillner and colleagues in the late 1970's and early 1980's. They demonstrated that the onset of swing phase activity in the spinal cat was dependent on the angle of the hip joint (Grillner and Rossignol 1978) and re-confirmed Sherrington's (1910a) observation that an afferent signal indicating limb position was involved in regulating the step cycle. The fact that sinusoidal extension / flexion of the hip joint could entrain the fictive locomotor rhythm when only innervation to the hip joint capsule and a number of small muscle acting around the joint remained (Andersson and Grillner 1983), further implicated a function of afferents signalling joint position. However, before inferring that hip joint afferents are directly involved in locomotion it must be established whether these afferents exhibit an appropriate pattern of activity during normal locomotor movements to warrant such a conclusion.

Exactly what parameters are conveyed by joint receptor afferents is a contentious issue. A number of researchers assert that joint afferents serve primarily as limit detectors, becoming active only when the joint is near the maximum range of motion (hip - Rossi and Grigg 1982; knee - Burgess and Clark 1969; Clark and Burgess 1975). On the other hand, some investigators report that activity in these afferents can indicate a wide range of joint positions (hip - Carli et al. 1979, 1981; knee - Ferrell 1980). Burgess and Clark (1969) found that only 5% of all knee joint afferent in the posterior articular nerve signaled intermediate joint angles, while Ferrell documented 20%. In both cases the majority of all afferents signaled flexion or extension near the physiological range of the joint. Similarly, Rossi and Grigg document that most hip afferents are activated by

movements which are outside the range of motion that normally occurs during locomotion in the cat. Thus, while a fairly broad range of joint positions could be signaled by joint receptor afferents, the majority indicate only extreme positions that are not relevant to normal locomotion.

1.2.4. Combined action of afferent feedback

The early commentaries made by neurophysiological pioneers, such as T. Graham Brown and Charles Sherrington, regarding the necessity of afferent feedback in the regulation of locomotion were based primarily on observations of stimulus and response. The more sophisticated techniques available today, have enabled researchers to document more complex reactions to various stimuli. Properties such as phase dependent reversal of cutaneous reflexes, state dependent reversal of tendon organ reflexes, and phase dependent modulation of muscle spindle afferent transmission have been shown to occur during locomotion. Understanding this highly regulated efficacy of afferent transmission is a primary goal of modern motor control research.

The fact that modulation occurs in many different reflex pathways suggests that regulation of locomotor activity occurs via multi-modal afferent feedback. There is the distinct possibility of large convergence of the ensemble afferent information generated during movement of the limb. For example, in non-walking preparations the group Ib inhibitory interneuron has been shown to receive convergent input from many sources (see Jami 1992), thus indicating that this reflex pathway does not simply reflect changes in muscle force. However, before investigations into the complexities of integrating multi-modal afferent feedback can occur, we must first understand the effects of the individual afferent systems.

1.3. Thesis Objectives.

1.3.1. Objective of Chapter 2:

Physiological response to absence of ground support during walking.

The role of sensory feedback during locomotor activity has often been investigated by introducing novel or increased levels of afferent stimuli and then monitoring any change in behavior or motor activity. The discovery of phase dependent reversal of cutaneous reflexes during walking [as described earlier] is a classic example of advances made using this methodology. **Chapter 2** of this thesis follows in this tradition making use of a novel gait perturbation to investigate sensory involvement in locomotion. The perturbation involves the sudden removal of ground support just prior to hindlimb foot contact during walking in the cat.

There are a number of origins for this particular type of gait perturbation. An early study on the behavioral responses to the unexpected absence of ground support during walking was performed on locusts in an effort to set up a paradigm for robotic locomotion (Pearson and Franklin 1984). It was observed that upon entering a hole at the end of swing phase the limb would re-initiate a flexion movement which would lift the limb out of the hole and allow it to "search" for new support. A separate unrelated set of studies, attempted to mimic the lack of ground support by anaesthetizing the foot pad in a walking cat in order to examine the effect of cutaneous afferent feedback on the EMG profile of the ankle extensor lateral gastrocnemius (Llewellyn et al. 1989; ***see also Engmerg 1969***). These experiments indicated that the cutaneous afferent discharge arising from ground contact did not noticeably affect the profile of LG activity. Given the lack of an anticipated result, the logical progression was to investigate how the total loss of proprioceptive discharge associated with ground contact affected the locomotor behavior and EMG activity.

Gorassini et al. (1994) designed a narrow runway with a trapdoor that opened unexpectedly under a hind leg during walking. It was observed that upon being extended into the hole, the limb rapidly hyper-flexed and was re-positioned in front of the obstacle. The results from this experiment demonstrated that, like cutaneous afferents, the

proprioceptive afferent discharge associated with ground contact does not influence the initial 50ms of LG EMG activity following ground contact. An interesting observation that arose from these experiments was that the latency of the flexion movement became faster with repeated exposure to the experimental situation, suggesting the possibility the animal was learning to respond to the hole. **Chapter 2** of this thesis investigates the potential role of supraspinal centers in producing the corrective response to the absence of ground support by comparing the response in intact and chronic spinal cats.

1.3.2. Objectives of Chapters 3 & 4:

Afferent regulation of stance phase duration.

The results from **Chapter 2** indicate that supraspinal centres act to decrease the latency and facilitate the vigor of the flexion response out of the hole. The precise structures responsible for the facilitation remain unknown, although inactivation of the motor cortex (Gorassini 1994) or the cerebellar nuclei (personal communication, M. Gorassini) does not change the corrective response. Elucidating the relevant ascending/descending spinal pathways and the supraspinal structures involved remains a potential area of research. However, during the completion of **Chapter 2** it became clear that the response to the absence of ground support also presented an excellent forum for investigating the afferent regulation of walking.

There are two interesting phenomena associated with the absence of ground support. There is an early initiation of swing phase activity of the limb entering the hole and a prolongation of stance phase activity in the contralateral limb. The fact that these responses were present in the chronic spinal animal (Chapter 2) indicated that afferent feedback, generated by the perturbation, acted on the spinal circuitry to modify the locomotor pattern. It was therefore hypothesized that if the level of afferent feedback could be manipulated as the limb encountered the hole then any change in the corrective response would provide further insight into the role of the afferent feedback during normal walking.

One postulate from Chapter 2 was that proprioceptive group I afferent feedback signaling loading of the limb acted to maintain extensor activity, and that when the limb

was in the hole the absence of that positive feedback allowed the early onset of flexion. This idea is tested in **Chapter 3** by electrically activating the large group I muscle afferents from various hindlimb extensors as the limb entered the hole. Another possible proprioceptive signal involved in generating the rapid onset of corrective flexion reaction could be generated by the rapid extension of the limb into the hole. In **Chapter 4**, it is investigated whether rapid stretch of flexor muscles, or electrical activation of flexor muscle afferents during locomotion would facilitate the transition from stance to swing phase, and facilitate the onset of flexion out of the hole.

1.3.3. Objective of Chapter 5:

Afferent regulation of extensor activity during stance phase.

A component of the corrective response to an absence of ground support is the prolongation of stance phase activity in the limb contralateral to the hole. Chapter 2 also showed that the level of motor activity in the contralateral quadriceps muscle distinctly increases during the corrective response in both intact and spinal animals. Furthermore, the results from Chapter 3 demonstrate that while extensor group I afferent feedback has a strong effect on the timing of the locomotor rhythm, afferent feedback is also important in generating the normal level of extensor muscle activity present during stance phase. In **Chapter 5** a variety of different experimental protocols are used in order to assess the degree to which muscle afferent feedback contributes to the generation of muscle activity.

1.4 References.

Akazawa, K., Aldgridge, J. W., Steeves, J. D., and Stein, R. B. Modulation of stretch reflexes druing locomotion in the mesencephalic cat. *J. Physiol.* 329:553-567, 1982.

Andersson, O. and Grillner, S. Peripheral control of the cat's step cycle: II. Entrainment of the central pattern generators for locomotion be sinusoidal hip movements during `fictive locomotion'. *Acta Physiol. Scand.* 118:229-239, 1983.

Angel, M. J., Guertin, P., Jimenez, I., and McCrea, D. A. Group I extensor afferents evoke disynaptic EPSPs in cat hindlimb extensor motoneurons during fictive locomotion. *J. Physiol.* 494:851-861, 1996.

Appenteng, K. and Prochazka, A. K. Tendon organ firing during active muscle lengthening in awake, normally behaving cats. *J. Physiol.* 353:81-92, 1984.

Brown, T. G. The intrinsic factors in the act of progression in the mammal. Proc. R. Soc. 84:308-319, 1911.

Burgess, P. R. and Clark, F. J. Characteristics of knee joint receptors in the cat. *J. Physiol.* 203:317-335, 1969.

Carli, G., Farabollini, F., Fontani, G., and Meucci, M. Solwly adapting receptors in cat hip joint. *J. Neurophysiol.* 42:767-778, 1979.

Carli, G., Fontani, G., and Meucci, M. Static characteristics of muscle afferents from gluteus medius muscle: comparison with joint afferents of hip in cats. *J. Neurophysiol.* 45:1085-1095, 1981.

Clark, F. J. and Burgess, P. R. Slowly adapting receptors in cat knee joint: can they signal joint angle? *J. Neurophysiol.* 38:1448-1473, 1975.

Cooper, S. The responses of the primary and secondary endings of muscle spindles with intact motor innervation during applied stretch. *J. Exp. Physiol.* 46:383-398, 1961.

Conway, B. A., Hultborn, H., and Kiehn, O. Proprioceptive input resets central locomotor rhythm in the spinal cat. *Exp. Brain Res.* 68:643-656, 1987.

Conway, B. A., Scott, D. T., Riddel, J. S., and Hadian, M. R. Effects of plantar nerve stimulation on the transmission of later reflexes in the decerebrate spinal cat. *J. Physiol.* 479P:145-146, 1994.

Crago, P. E., Mathews, P. B. C., and Rymer, W. Z. Sampling of total muscle force by tendon organs. *J. Neurophysiol.* 47(6):1069-1083, 1982.

Duysens, J. Reflex control of locomotion as revealed by stimulation of cutaneous afferents in spontaneously walking premamillary cats. *J. Neurophysiol.* 40:737-750, 1977.

Duysens, J. and Pearson, K. G. Inhibition of flexor burst generation by loading ankle extensor muscles in walking cats. *Brain Res.* 187:321-332, 1980.

Duysens, J. and Pearson, K. G. The role of cutaneous afferents from the distal hindlimb in the regulation of the step cycle of thalamic cats. *Exp. Brain Res.* 24:245-255, 1976.

Duysens, J. and Stein, R. B. Reflexes induced by nerve stimulation in walking cats with implanted cuff electrodes. *Exp. Brain Res.* 32:213-224, 1978.

Duysens, J., Trippel, M., Horstmann, G. A., and Dietz, V. Gating and reversal of reflexes in ankle muscles during human walking. *Exp. Brain Res.* 82:351-358, 1990.

Ferrell, W. R. The adequacy of stretch receptors in the cat knee joint for signalling joint angle throughout a full range of movement. *J. Physiol.* 299:85-99, 1980.

Forssberg, H. Stumbling corrective reaction: a phase dependent compensatory reaction during locomotion. *J. Neurophysiol.* 42:936-953, 1979.

Forssberg, H., Grillner, S., and Halbertsma, J. M. The locomotion of the low spinal cat. I. Coordination within a hindlimb. *Acta Physiol. Scand.* 108:269-281, 1980.

Forssberg, H., Grillner, S., and Rossignol, S. Phase dependant reflex reversal during walking in chronic spinal cats. *Brain Res.* 85:103-107, 1975.

Forssberg, H., Grillner, S., and Rossignol, S. Phasic gain control of reflexes from the dorsum of the paw during spinal locomotion. *Brain Res.* 132:121-139, 1977.

Freusburg, A. Reflexbewegungen beim Hunde. Pflugers. *Arch. ges. Phsyiol.* 9:358-391, 1874.

Gladden, M. H. Muscle receptors in mammals. In: *Advances in comparative and environmental physiology*, Vol.10, Berlin Heidelber: Springer-Verlag, 1992, p. 281-302.

Gorassini, M. A. The control and use of proprioceptive information during purposeful movement. Ph.D. thesis. University of Alberta , 1994.

Gorassini, M. A., Prochazka, A. K., Hiebert, G. W., and Gauthier, M. J. A. Corrective responses to loss of ground support during walking I. Intact cats. *J. Neurophysiol.* 71:603-610, 1994.

Goslow, G. E., Reinking, R. M., and Stuart, D. G. The cat step cycle: Hindlimb joint angles and muscle lengths during unrestrained locomotion. *J. Morph.* 141:1-42, 1973.

Gossard, J. -P. , Brownstone, R. M. , Barajon, I. , and Hultborn, H. Transmission in a locomotor-related group Ib pathway from hindlimb extensor muscles in the cat. *Exp. Brain Res.* 98:213-228, 1994.

Gregory, J. E. and Proske, U. The responses of Golgi tendon organs to stimulation of different combinations of motor units. *J. Physiol.* 295:251-262, 1979.

Grillner, S. Locomotion in the spinal cat. In: *Control of posture and locomotion*. edited by R. B. Stein, K. G. Pearson, R. S. Smith and J. B. Redford. New York: Plenum Press, 1973, p. 515-535.

Grillner, S. and Rossignol, S. On the initiation of the swing phase of locomotion in chronic spinal cats. *Brain Res.* 146:269-277, 1978.

Grillner, S. and Zangger, P. How detailed is the central pattern generation for locomotion? *Brain Res.* 88:367-371, 1975.

Grillner, S. and Zangger, P. On the central generation of locomotion in the low spinal cat. *Exp. Brain Res.* 341:241-261, 1979.

Guertin, P., Angel, M. J., Perreault, M. -C., and McCrea, D. A. Ankle extensor group I afferents excite extensors throughout the hindlimb during MLR-evoked fictive locomotion in the cat. *J. Physiol.* 487(1):197-210, 1995.

Halbertsma, J. M. The Stride Cycle of the Cat: The modelling of locomotion by computerized analysis of automatic recordings. *Acta Physiol. Scand.* (Suppl) 521:1-75, 1983.

Horcholle-Bossavit, G., Jami, L., Petit, J., Vejsada, R., and Zytnicki, D. Ensemble discharge from golgi tendon organs of cat peroneus tertius muscle. *J. Neurophysiol.* 64:813-821, 1990.

Houk, J. C. and Henneman, E. Responses of golgi tendon organs to active contractions of the soleus muscle of the cat. *J. Neurophysiol.* 30:466-481, 1967.

Jami, L. Golgi tendon organs in mammalian skeletal muscle: Functional properties and central actions. *Physiol. Review* 72:623-666, 1992.

Jankowska, E. Interneuronal Relay in Spinal Pathways from Propriocetors. *Progress in Neurobiology* 38:335-378, 1992.

Jankowska, E., Jukes, M. G. M., Lund, S., and Lundberg, A. The effect of DOPA on the spinal cord: 5. Reciprocal organization of pathways transmitting excitatory action to alpha motoneurones of flexors and extensors. *Acta Physiol. Scand.* 70:369-3386, 1967a.

Jankowska, E., Jukes, M. G. M., Lund, S., and Lundberg, A. The effect of DOPA on the spinal cord: 6. Half-centre organization of interneurones transmitting effects from the flexor reflex afferents. *Acta Physiol. Scand.* 70:389-402, 1967b.

Kuffler, S. W., Hunt, C. C., and Quilliam, J. P. Function of medullated small-nerve fibres in mammalian ventral roots: efferent muscle spindle innervation. *J. Neurophysiol.* 14:29-54, 1951.

Laporte, Y. and Lloyd, D. P. C. Nature and significance of the reflex connections established by large afferent fibers of muscular origin. *Am. J. Physiol.* 169:609-621, 1952.

Leksell, L. The action potential and excitatory effects of the small ventral root fibres to skeletal muscle. *Acta Physiol. Scand.* 21:1-84, 1945.

Llewellyn, M., Prochazka, A. K., and Trend, P. St. J. Control of ankle extensor activation prior to and after foot contact in cat step cycle. *J. Physiol.* 418:146P, 1989.

Loeb, G. E. Somatosensory unit input to the spinal cord during normal walking. *Can. J. Physiol. Pharmacol.* 59:627-635, 1981.

Loeb, G. E. and Hoffer, J. A. Activity of spindle afferents from cat anterior thigh muscles. II. Effects of fusimotor blockade. *J. Neurophysiol.* 54:565-577, 1985.

Lundberg, A. Half-centres revisited. In: *Regulatory Functions of the CNS. Motion and Organizational Principles*, edited by J. Szentagothai, M. Palkovits and J. Jamori. Pergamon Press, p. 155-167, 1980.

Matthews, P. B. C. The differentiation of two types of fusimotor fibre by their effects on the dynamic responses of muscle spindle primary endings. *Q. J. Exp. Physiol.* 47:324-333, 1962.

McCrea, D. A., Shefchyk, S. J., Stephens, M. J., and Pearson, K. G. Disynaptic group I excitation of synergistic ankle extensor motoneurones during fictive locomotion. *J. Physiol.* 487(2):527-540, 1995.

Murphy, P. R., Stein, R. B., and Taylor, J. L. Phasic and tonic modulation of impulse rates in gamma-motoneurons during locomotion on premammilary cats. *J. Neurophysiol.* 52(2):228-243, 1984.

Pearson, K. G. and Collins, D. F. Reversal of the influence of group Ib afferents from plantaris on activity in medial gastrocnemius muscle during locomotor activity. *J. Neurophysiol.* 70:1009-1017, 1993.

Pearson, K. G. and Duysens, J. Function of segmental reflexes in the control of stepping in cockroaches and cats. In: *Neural control of locomotion*, edited by R. M. Herman, S. Grillner, P. S. G. Stein and D. G. Stuart. New York: Plenum Press, 1976, p. 519-538.

Pearson, K. G., Fourtner, C. R., and Wong, R. K. S. Nervous control of walking in the cockroach. In: *Control of posture and locomotion*, edited by R. B. Stein, K. G. Pearson, R. S. Smith and J. B. Redford. New Yourk: Plenum Press, 1973, p. 495-514.

Pearson, K. G. and Franklin, R. Characteristics of leg movements and patterns of coordination in locusts walking on rough terrain. *Int. J. Robotics* 3:101-112, 1984.

Pearson, K. G., Ramirez, J. M., and Jiang, W. Entrainment of the locomotor rhythm by group Ib afferents from ankle extensor muscles in spinal cats. *Exp. Brain Res.* 90:557-566, 1992.

Perret, C. Analyse des mécanismes d'une activité de type locomoteur chez le chat (PhD Thesis). Paris: Université de Paris VI, 1973.

Philippson, M. L'autonomie et la centralization dans le système nerveux des animaux. *Tav. Lab. Phsyiol. Inst. Solvay (Bruxelles)* 7:1-208, 1905.

Prochazka, A. K., Sontag, K. H., and Wand, P. Motor reactions to perturbations of gait: proprioceptive and somesthetic involvement. *Neuroscience Letters* 7:35-39, 1978.

Prochazka, A. K., Trend, P. St. J., Hulliger, M., and Vincent, S. Ensemble proprioceptive activity in the cat step cycle: towards a representative look-up chart. In: *Progress in Brain Research. Afferent Control of Posture and Locomotiom*, edited by J. H. J. Allum and M. Hulliger. Amsterdam: Elsevier, 1989, p. 61-74.

Rossi, G. and Grigg, P. Characteristics of hip joint mechanoreceptors in the cat. *J. Neurophysiol.* 47:1029-1042, 1982.

Severin, F. V. The role of the gamma motor system in the activation of the extensor alpha motor neurones during controlled locomotion. *Biophysics* 15:1096-1102, 1970.

Shefchyk, S. J., Stein, R. B., and Jordan, L. M. Synaptic transmission from muscle afferents during fictive locomotion in the mesencephalic cat. *J. Neurophysiol.* 51:986-997, 1984.

Sherrington, C. S. Flexion-reflex of the Limb, crossed extension-reflex, and reflex stepping and standing. *J. Physiol.* 40:28-121, 1910a.

Sherrington, C. S. Remarks on the reflex mechanism of the step. *Brain* 33:1-25, 1910b.

Stephens, J. A., Reinking, R. M., and Stuart, D. G. Tendon organs of cat medial gastrocnemius: Reponses to active and passive forces as a function of muscle length. *J. Neurophysiol.* 38:1217-1231, 1975.

Stein, R. B. and Capaday, C. The modulation of human reflexes during functional motor tasks. *TINS* 11:328-332, 1988.

Taylor, J. L., Stein, R. B., and Murphy, P. R. Impulse rates and sensitivity to stretch of soleus muscle spindle afferent fibers during locomotion in premammillary cats. *J. Neurophysiol.* 53(2):341-360, 1985.

Viala, D. and Buser, R. Modalites d'obtention de rythmes locomoteurs chez le lapin par traitements pharmacologiques (DOPA, 5-HTP, d'amphetamine). *Brain Res.* 35:151-165, 1971.

Wand, P., Prochazka, A. K., and Sontag, K. H. Neuromuscular responses to gait perturbations in freely moving cats. *Exp. Brain Res.* 38:109-114, 1980.

Wetzel, M. C. and Stuart, D. G. Ensemble characteristics of cat locomotion and its neural control. *Progress in Neurobiology* 7:1-98, 1976.

Yang, J. F. and Stein, R. B. Phase-dependant reflex reversal in human leg muscles during walking. *J. Neurophysiol.* 63:1109-1117, 1990.

CHAPTER 2.

Corrective responses to the absence of ground support during walking in intact and spinal cats.*

2.1 Introduction.

One approach for determining the function of afferent feedback from peripheral receptors during walking is to perturb the step cycle and monitor electromyographic (EMG) responses in leg muscles. In humans, for example, sudden accelerations or decelerations of a treadmill evoke characteristic, compensatory responses in leg muscles that appear to be triggered by input from group II muscle afferents (Berger et al. 1984; Dietz et al. 1985,1987). Corrective reactions to tactile stimulation of the paws of cats have also been extensively studied (Forssberg 1979; Wand et al. 1980; Drew and Rossignol 1987). A general issue that arises from these types of studies is whether or not the corrective reactions are organized at a spinal or supraspinal level. Current evidence indicates that the stumbling-corrective reaction in the cat is mediated primarily via spinal reflex pathways that are strongly modulated in a phase-dependent manner by the locomotor rhythm generator. Moreover, differences in the effectiveness of these reflex pathways in intact, mesencephalic and chronic spinal cats have shown that the spinally-organized reactions are modulated by supraspinal structures (Forssberg 1979).

To further investigate corrective reactions during walking in intact cats the behaviour of the hind legs when one foot unexpectedly enters a hole in the supporting surface has been examined (Gorassini et al. 1994). In this situation the foot is quickly

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removed from the hole by strong flexion at the knee and ankle, while support of the hindquarters is maintained by a prolongation of stance in the contralateral limb. EMG recordings from hind leg muscles have shown that the latency to the onset of the flexor activity responsible for withdrawing the foot from the hole is in the range of 35ms to 65ms (Gorassini et al. 1994). These relatively long latencies to the onset of changes in the motor pattern raises the possibility that the flexor burst activity required to remove the foot from the hole is initiated via supraspinal pathways. Thus the main objective of this investigation was to assess the contribution of supraspinal structures by comparing the responses of intact and chronic spinal cats to the entry of one of their hind legs into a hole cut in the treadmill belt upon which they were stepping. The data show that the flexion response of the leg entering the hole is more rapid in onset and larger in amplitude in the intact animal, indicating that supraspinal pathways enhance the flexion response by modulating the spinal system that normally produces swing-phase flexor activity. The data also indicate that proprioceptive feedback can strongly regulate the timing and amplitude of EMG activity during walking.

2.2 Methods.

The primary objective of this study was to compare the reactions in the hind legs of intact and chronic spinal cats when one hind foot failed to contact the supporting surface at the end of the swing phase of walking. Because stepping of the hind legs in chronic spinal cats is bipedal and can only be elicited reliably on a treadmill, it was important to examine intact animals under similar conditions.

2.2.1. Bipedal stepping in intact cats.

Six cats were trained with food rewards to step with their hind legs on a moving treadmill belt. Four hours prior to each training session, food was removed from the home cage. During training, food was presented, at mouth level, while the hind legs were stepping on the moving treadmill. The forelegs were supported on a platform 2cm above the treadmill belt. Animals were trained once per day, each training session lasting 10-15

minutes. Within a few days the animals learned to continuously step bipedally for many minutes and would adapt their rate of stepping to wide variations in treadmill speed (0.1 to 1m/s).

2.2.2. Stepping in chronic spinal cats.

Three of the animals that had been trained to step bipedally and three other animals were spinalized at the T12/13 level under aseptic conditions. Briefly, the spinal cord was exposed by ablating the muscle tissue along the spine, and a partial laminectomy of the T12 vertebrae. The cord was then transected with a pair of scissors. Successful transection of the cord was confirmed by observing the gap produced between the stumps of the cord, and by packing the transection sight with a blood coagulant (SURGICEL hemostat; Johnson & Johnson Medical Inc.) For one week postoperatively, under veterinary supervision, each animal received an antibiotic (1cc Ayercillin per day) and, if required, an analgesic (buprenorphine, 0.005 - 0.01 mg/kg). The bladder was emptied using a catheter for the first week following spinalization and subsequently expressed manually twice a day.

Locomotor training was commenced a few days after spinalization. The animal was positioned so that its hindlimbs were situated on a treadmill while the forelegs rested on a stationary plate. Movements of the hind legs were evoked by pinching the skin of the perineal region, and the posture of the animal was adjusted so that the legs thrust against the moving treadmill belt during leg extension. Balance and weight support was assisted by holding the tail. In five of the six animals the strength and coordination of the stepping movements progressively improved over a period of 2 to 3 weeks to the point where stepping in the hind legs could occur in the absence of perineal skin stimulation and be sufficiently powerful to support completely the weight of the hindquarters. Training sessions lasted about 20 minutes and were performed twice daily. Food rewards were given to three of the animals (those previously used in the intact group) to minimize distractions.

2.2.3. EMG implants.

Once the six intact cats had been trained to walk bipedally, pairs of electrodes (Cooner Wire Company, AS632) were sutured into various flexor and extensor muscles of the hind legs: lateral gastrocnemius (LG), vastus lateralis (VL), iliopsoas (IP), semitendinosus (ST) and tibialis anterior (TA). The wires were led subcutaneously up to the animal's head where they were attached to a multi-pin connector terminal. Two stainless steel screws were placed into the parietal cranium.The connector was securely mounted to the skull and the screws with dental acrylic. Connections to the recording amplifiers were made via a long flexible cable attached to the headpiece. Three of these intact animals were subsequently spinalized and the same electrode array was used to monitor EMG activity during the recovery of stepping and during experimental trials when the left foot entered the hole in the treadmill. In the other three chronic spinal animals, EMG recordings were made percutaneously from a limited number of muscles (usually 4) on the day of the recording session.

2.2.4. Response to loss of ground support.

When either an intact cat had been trained to step bipedally or a chronic cat had been trained to step with weight support of the hindquarters during perineal stimulation, the treadmill belt was changed to one containing a hole (10 cm x 30 cm) on the left side. This resulted in the left hind leg occasionally stepping into the hole. The entry of the paw into the hole was monitored by an array of infrared light-emitting diodes directed across the hole onto an array of phototransistors (spacing 1.0 cm), located 1.5cm below the top surface of the treadmill belt. This device will be referred to here and in **Chapter 3** as the "light-fence". Disruption of any part of the light fence resulted in a 5V pulse for the duration of light obstruction. Because of the position of the light fence below the treadmill belt, activation of the light fence was slightly delayed from when the paw was level with the top of the treadmill belt. From measurements of the foot velocity at the end of the swing phase from video recordings it was estimated that the interval from the time the foot entered the hole to activation of the light fence was approximately 30ms.

During each testing session, up to 20 successful encounters with the hole were recorded. A trial was judged to be successful if the paw entered the hole without contacting the rim. Trials where the limb did make contact were discarded because of the possible activation of cutaneous afferents. All animals were fed during the testing sessions in the same manner as during training. An important parameter during testing was the treadmill speed. This was usually kept low (0.2 to 0.3m/s) so that the contralateral (right) leg could support the hindquarters during the period that the left leg was in the hole. If the left foot was not withdrawn quickly to regain support on the treadmill and/or the right leg initiated swing before the left leg support had been achieved, the animal was prevented from collapsing by supporting the abdomen by hand (intact animals) or lifting the hindquarters by the tail (spinal animals). During testing in spinal animals, the tail was held to maintain balance of the hindquarters but not to support the animal's weight.

2.2.5. Data recording and analysis.

Leg movements were monitored using a video recorder (30 frames/s, shutter speed 1ms). Surface markers on the iliac crest, greater trochanter and lateral epicondyle of the femur, lateral malleolus, and metatarso-phalangeal joint were used to derive relative joint angles. The EMG signals were high (100Hz) and low (10kHz) pass filtered and amplified (x 1-10k). The signal was then recorded, along with the output from the light fence, on VHS tape with a Vetter Pulse Code Modulation recorder (Model 4000A).

All data analysis was done off-line. A hard copy of the data was displayed on a Gould ES1000 electrostatic chart recorder. Selected sequences of EMG records were rectified and low-pass filtered (50-100Hz) and stored on computer disc using the Axotape data acquisition system (Axon Instruments) installed in an Express 80 486 microcomputer. The sampling rate for each channel was 700Hz. Custom written programs were used to average EMG records and to establish the timing of events relative to the triggering of the light fence signal.

. A frame-by-frame analysis of the video recordings was used to determine the time of onset (to within 33ms) of the first visible flexion movement at the hip, knee or

ankle following the entrance of the limb into the hole. Joint angles and limb position in individual video frames were analyzed using Video-Blaster software (Creative Labs, Inc.) and custom written programs.

2.3. Results.

The responses to stepping into the hole in the treadmill belt were recorded in six intact and five chronic spinal cats. Three of the intact animals were subsequently spinalized and thus included in the spinal group.

2.3.1. Limb movements during corrective reaction to absence of ground support.

All the intact animals quickly lifted their foot from the hole and, provided the treadmill speed was low, maintained support of the hindquarters by prolonging the stance phase of the contralateral leg (Fig. 2.1). These responses to the absence of ground support closely resembled those made by intact animals when walking slowly on a walkway containing a trap door (Gorassini et al. 1994). The withdrawal of the paw from the hole was rapid in onset. The latency from the time the foot entered the hole to the beginning of leg flexion measured from the video recordings was in the range of 66 to 165ms, i.e. flexion at hip, ankle or knee was detected in the third to fifth frame after the foot entered the hole. Histograms showing the distribution of the latencies for two intact animals are shown in Fig. 2.2A and B (data from the other four animals were similar). The data in each of these histograms were obtained during a single test period.

Chronic spinal cats also flexed their leg soon after the foot entered the hole in the treadmill, but compared to intact animals the latencies to the onset of this response were longer and more variable (range 130 to 350ms). Histograms of latencies observed in two spinal animals are shown in Fig. 2.2C and D. On average the latency until a flexion response in spinal animals was about 4 frames longer (>130ms) than the mean latency in intact animals.

Another clear difference in the responses of intact and spinal animals was observed in the kinematics of the flexion movements initiated after the foot entered the

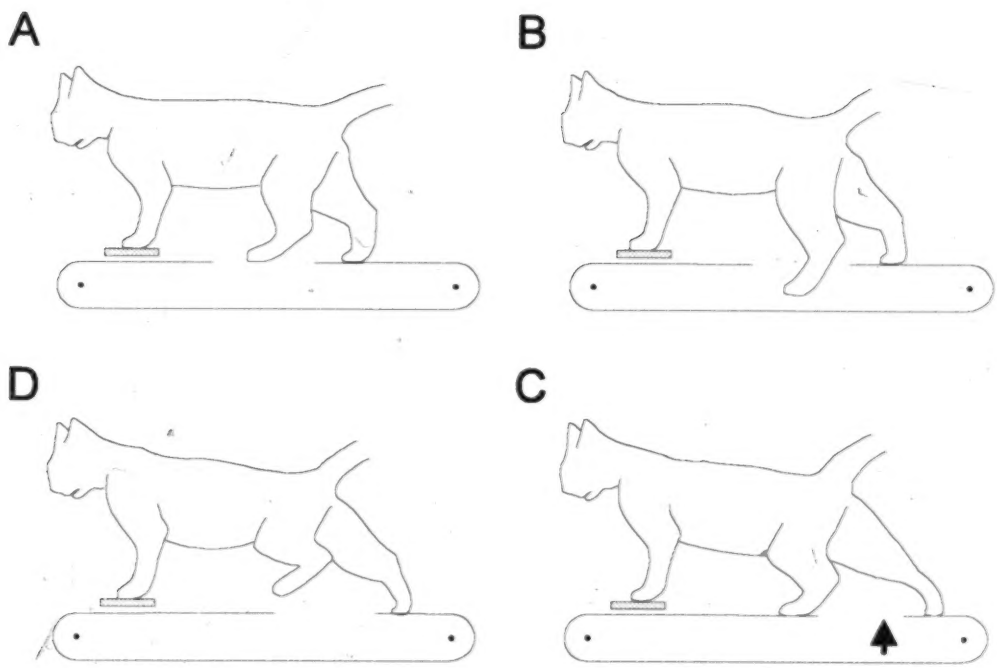

Figure 2.1. Drawings showing the corrective flexion reaction in response to one hindlimb encountering a hole in the supportive surface. Intact and chronic spinal cats stepped bipedally with their hind legs on a treadmill while standing with their forelegs on a stationary platform. The belt of the treadmill contained a hole on the left side. This sequence of drawings shows the positions of the hind legs in an intact animal when A) the left paw entered the hole, B) the left leg began to flex and withdraw from the hole, C) the left leg was maximally flexed, and D) the left paw regained support on the treadmill belt (note the large extension of the right leg, the arrowhead indicating the position of right paw at the stance to swing transition when the left leg did not enter the hole).

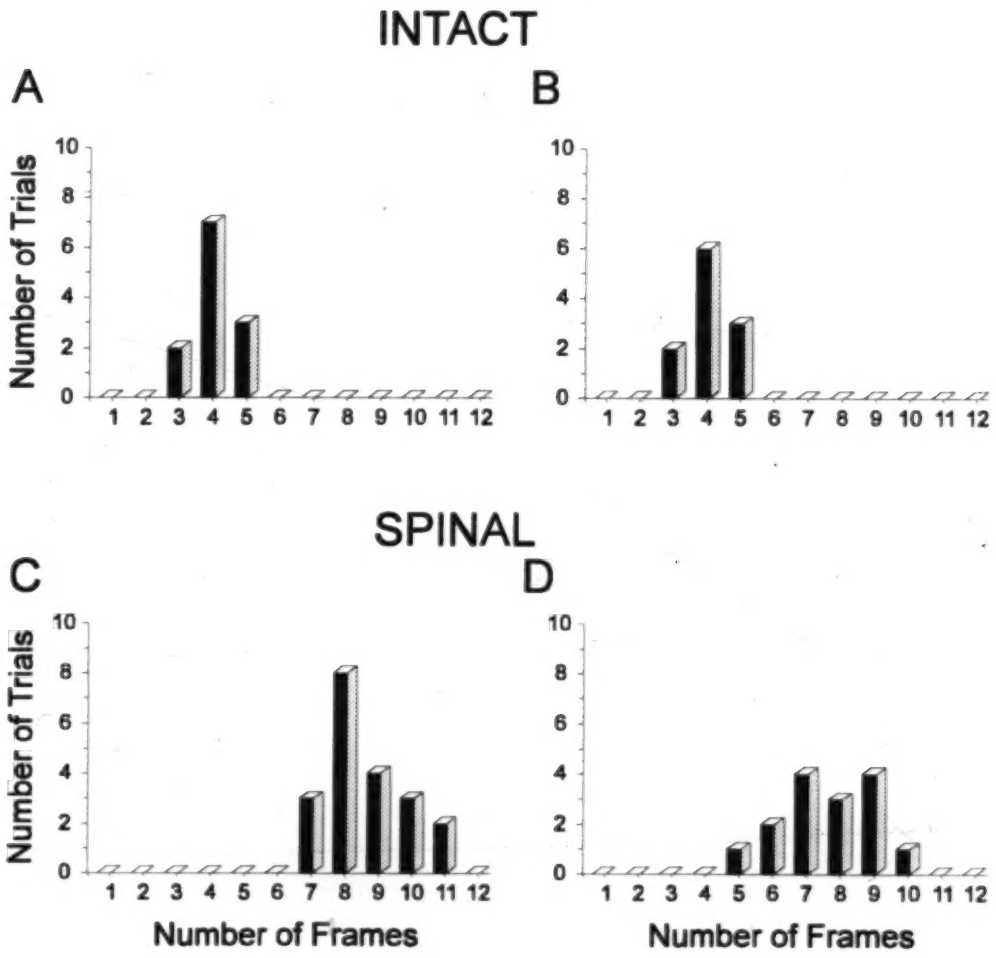

Figure 2.2. Histograms showing the latency of the onset of leg flexion after the paw of the left leg entered the hole in the treadmill. A,B - intact animals, C,D - spinal animals. The latency is expressed as the number of video frames (33ms/frame) from the time the paw entered the hole until the frame showing the first observable flexion movement at either the hip, knee or ankle. Each histogram represents data from a different animal during a single test session. On average the minimum latency of the

hole. In intact animals the flexion of the limb out of the hole was exaggerated compared to normal swing phase movements (Figs. 2.3A and 2.4). The paw was lifted high above the treadmill surface, whereas during swing the limb is flexed just sufficiently to clear the surface. The result of this strong flexion movement was that the foot almost always regained support on the treadmill. By contrast, the flexion movements in chronic spinal animals were weak and usually not sufficient to lift the paw above the level of the treadmill belt. Occasionally, the leg would then re-extend into the hole, to be succeeded by a second flexion movement. Even on those occasions that the first flexion led to the regaining of support on the treadmill belt, it was clear that the response was qualitatively different from that occurring in intact animals. Figure 2.3B shows an example in which foot support was regained after the first flexion movement. Note the weak elevation of the paw above the treadmill belt compared to the elevation achieved in an intact animal (Fig. 2.3A).

The quantitative features of the joint movements during swing and during the corrective response were determined from plots of the joint angles versus time in three intact and two spinal animals (see examples in Fig. 2.4). In the three intact animals the average value of the peak flexion at the knee increased by 6° (n=10), 9° (n=6) and 16° (n=5) relative to peak flexion during swing, while peak flexion at the ankle correspondingly increased by 19°, 16° and 23°. In the spinal animals, by contrast, there was no obvious increase in peak flexion at either the knee or the ankle joints. Another difference between the two groups of animals was that hip extension at the onset of the corrective response was noticeably less (mean of 20°, n=21) than at the onset of the normal swing phase in intact animals, whereas in spinal animals the hip extension at the beginning of the response was usually larger than at the onset of swing phase (range 0 to 14°). In general, the corrective responses with the longest latencies were associated with the larger values of hip extension (not shown).

2.3.2. Muscle activity during the corrective reaction.

EMG recordings revealed a characteristic pattern of motor activity in response to the paw entering the hole (Fig. 2.5). In the intact animal, LG (an ankle extensor) activity

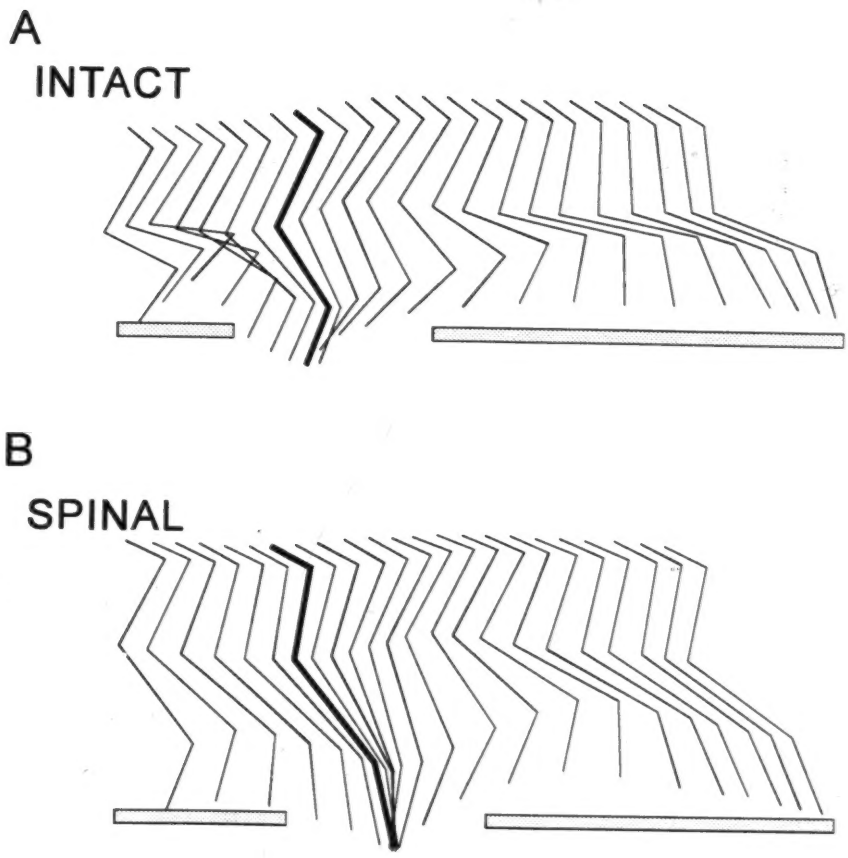

Figure 2.3. Stick figures showing the movement of the left leg during normal swing phase, extension into the hole, and the corrective response lifting the foot from the hole. A - intact animal, B - spinal animal. The thicker trace represents the onset of the corrective flexion response. Note the strong flexion of the limb during the corrective flexion response in the intact animal as compared to the spinal animal. For clarity, sequential traces in the figures have been shifted to the left by the distance moved by the treadmill belt. The time interval between each trace is 33 ms.

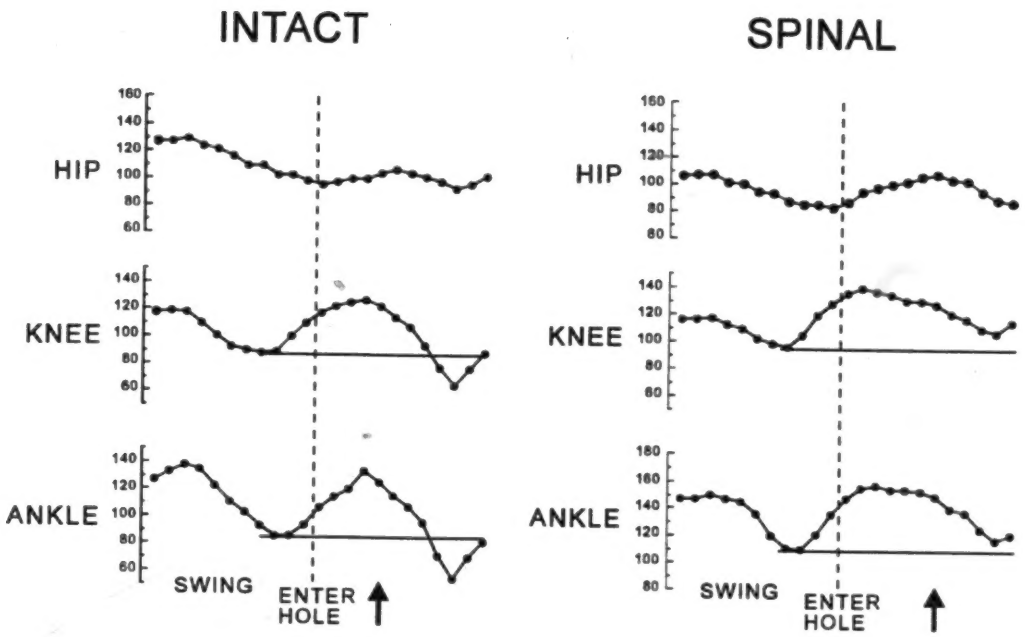

Figure 2.4. Plots showing the joint angles of the hip (top), knee (middle) and ankle (bottom) during the swing phase of walking and during the subsequent corrective response in an intact and a spinal animal. The plots begin at the moment of foot lift off and end when the foot regained support on the treadmill belt. The vertical line indicates the point at which the limb would normally have contacted the surface of the treadmill. The onset of the corrective response is indicated by the arrow. The horizontal lines indicate peak flexion of the knee and ankle during swing.

commenced about 70ms before the foot entered the hole and ceased abruptly 60 to 100ms after foot entry. This burst of activity in LG resembled the initial part of the LG bursts generated during stance when walking bipedally (Fig. 2.6A) and during quadrupedal walking (see Figs. 2 & 3 in Gorassini et al. 1994). When the foot entered the hole there was little activity in LG for approximately 150ms after the initial burst. It was during this period that the flexors were maximally active (Fig. 2.5A, top two traces). The knee extensor VL demonstrated a similar pattern of activity. A small burst of activity occurred just prior to limb contact. The EMG activity then ceased as the limb flexors became active (Fig. 2.5A).

A qualitatively similar pattern of extensor activity was seen in spinal animals (Figs. 2.5B and 2.6C, D). Activity in LG commenced about 60ms prior to the foot entering the hole and declined abruptly soon after entry. This initial burst of activity was similar to that occurring around the time of ground contact during stance (Fig. 2.6C). The decline in this initial burst after the foot entered the hole was followed by a period of either inactivity or low level activity lasting for about 250ms. The latter part of this period corresponded to the generation of burst activity in flexors (Fig. 2.5B). A similar pattern of activity was seen in VL (Fig. 2.6D).

In both intact and spinal cats bursts of activity occurred in ipsilateral flexor muscles shortly after the limb entered the hole (see examples for ST and IP in Figs. 2.5 and 2.7). These bursts of activity in flexors were associated with the flexion of the limb out of the hole. Although the general features of the patterns of activity in flexors during the corrective response were similar in intact and spinal animals, a number of consistent differences in the timing and amplitude were observed. One of the most noticeable was that the onset of flexor activity was delayed in spinal animals. The latency was in the range of 40 to 80ms in intact animals and 100 to 250ms in spinal animals (relative to the estimated time the foot entered the hole). The difference corresponded to the behavioral observation that the time of onset in spinal animals was delayed (Fig. 2.2).

Another difference was that in intact animals the magnitude of the bursts in IP during the corrective flexion reaction was larger relative to that of the swing phase IP bursts, while in spinal animals, only a slight increase in relative magnitude was observed

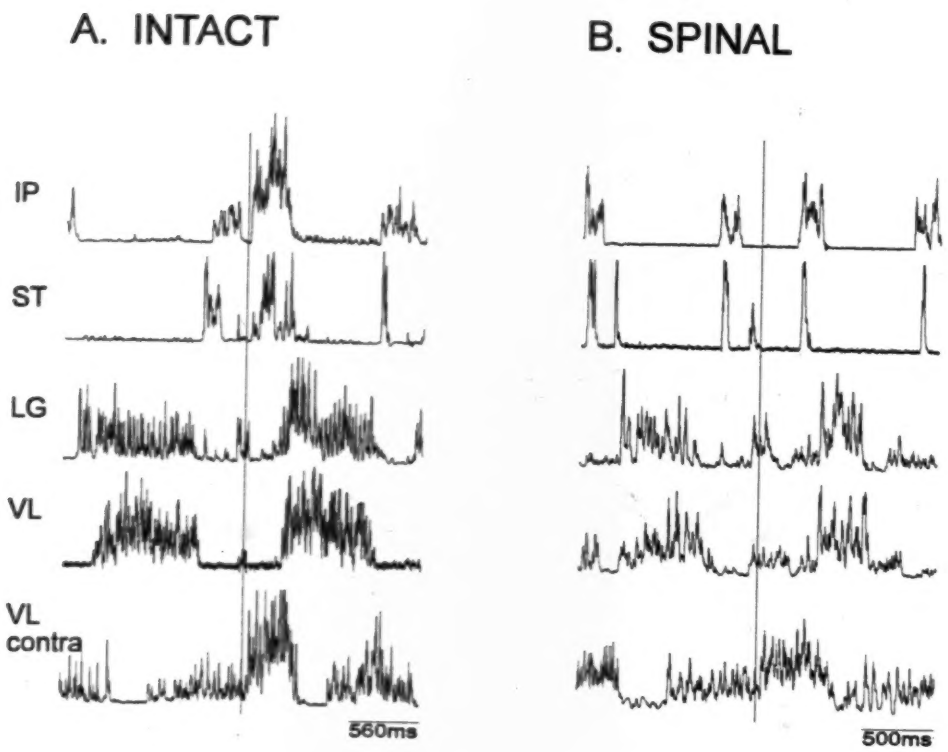

Figure 2.5. Rectified EMG records illustrating the characteristic muscle activity occurring when an intact cat (A) and a chronic spinal cat (B) encounter a hole in the treadmill belt. The vertical lines indicate the times that the paw passed through the light fence. The limb is estimated to have passed beneath the plane of the treadmill belt 30ms prior to the light fence signal. IP - iliopsoas, ST - semitendinosus, LG - lateral gastrocnemius, VL - vastus lateralis.

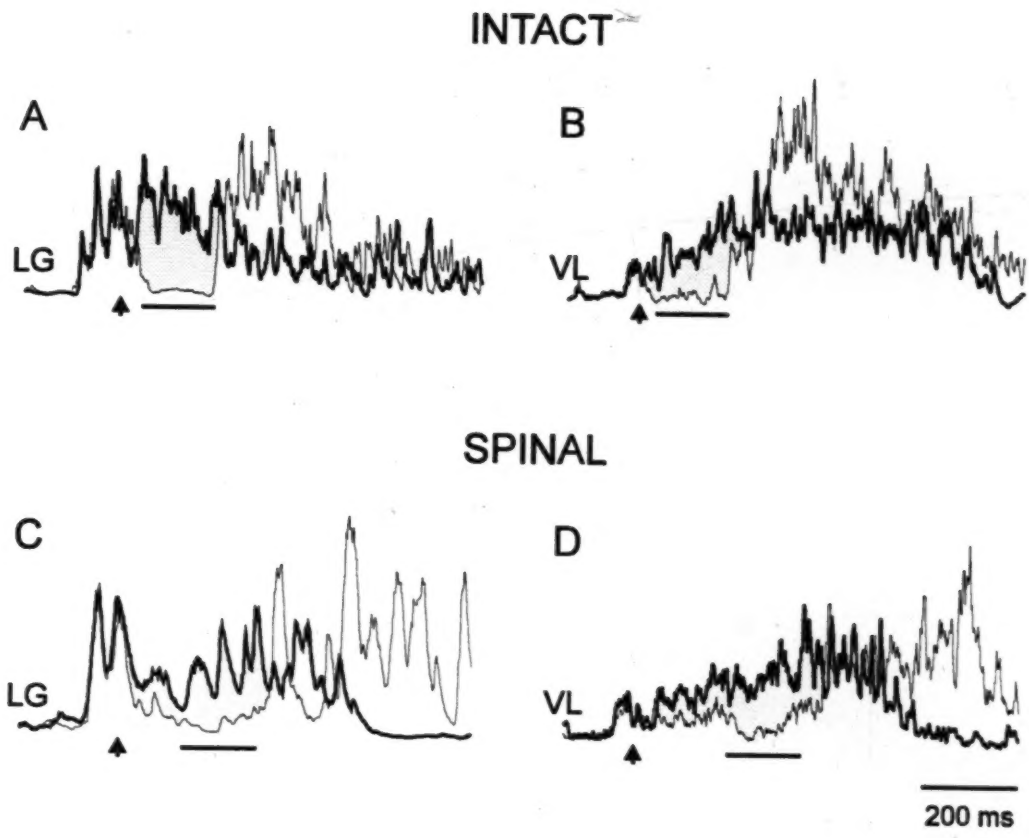

Figure 2.6. Rectified and averaged (n=4) EMGs recorded from the left lateral gastrocnemius (A,C) and vastus lateralis (B,D) muscles in an intact cat (A,B) and a chronic spinal cat (C,D). The thick traces represent the EMG activity during normal stance. The thin traces are the EMG patterns during trials where the left limb entered the hole. The arrows indicate the times the paw passed through the light fence and the horizontal bars indicated the periods of inactivity associated with flexor burst activity. The shaded areas illustrate the large decrease in extensor activity associated with the limb entering the hole.

(Figs. 2.5B and 2.7C,D). Also the magnitude of the bursts in ST during the corrective response in intact animals was quite variable. In some trials it was noticeably larger than the swing-phase bursts, while in others it was of similar magnitude. By contrast, in spinal cats the magnitude of ST bursts during the corrective response usually did not differ markedly from that observed during swing (Figs. 2.5 and 2.7C,D).

Finally, a difference was observed in the profile of the flexor bursts. In intact animals the magnitude of the bursts in IP, and occassionally ST, often increased noticeably during the burst (see arrow in Fig. 2.7B). The timing of the onset of this increase in activity was variable and in the range of 130 to 180ms. This augmentation of the flexor bursts was not observed in spinal animals, nor did it occur during the swing phase of stepping in either group of animals.

It is also important to note that some features of the flexor bursts during the corrective response were similar in intact and spinal animals. One was that in both groups of animals the durations of the bursts in IP were increased relative to the durations of the IP bursts during swing (Fig. 2.7). Another was that the onset of ST activity followed the onset of IP activity during the corrective response (Fig. 2.7) (note that ST activity usually precedes IP during swing in the intact cat). A third was that the ST bursts were shorter than the IP bursts, and ST often became weakly active at the time extensor activity resumed. This second, smaller burst of ST activity at the onset of stance is a characteristic feature of this muscle's activity pattern during walking (Fig. 2.7A,C).

2.3.3. Corrective response of the limb contralateral to the hole.

At low treadmill speeds (<0.4m/s) intact cats maintained support of their hindquarters during the corrective response by prolonging the stance phase of the contralateral leg (Figs. 2.1 and 2.8). This resulted in the contralateral leg extending further than normal. At higher treadmill speeds the flexion of the contralateral leg began while the ipsilateral foot was in the hole, thus causing a collapse of the hindquarters. Because this disturbed the subsequent behaviour of the animal (manifested by a reluctance to walk with a stable bipedal pattern in the center of the treadmill), the speed

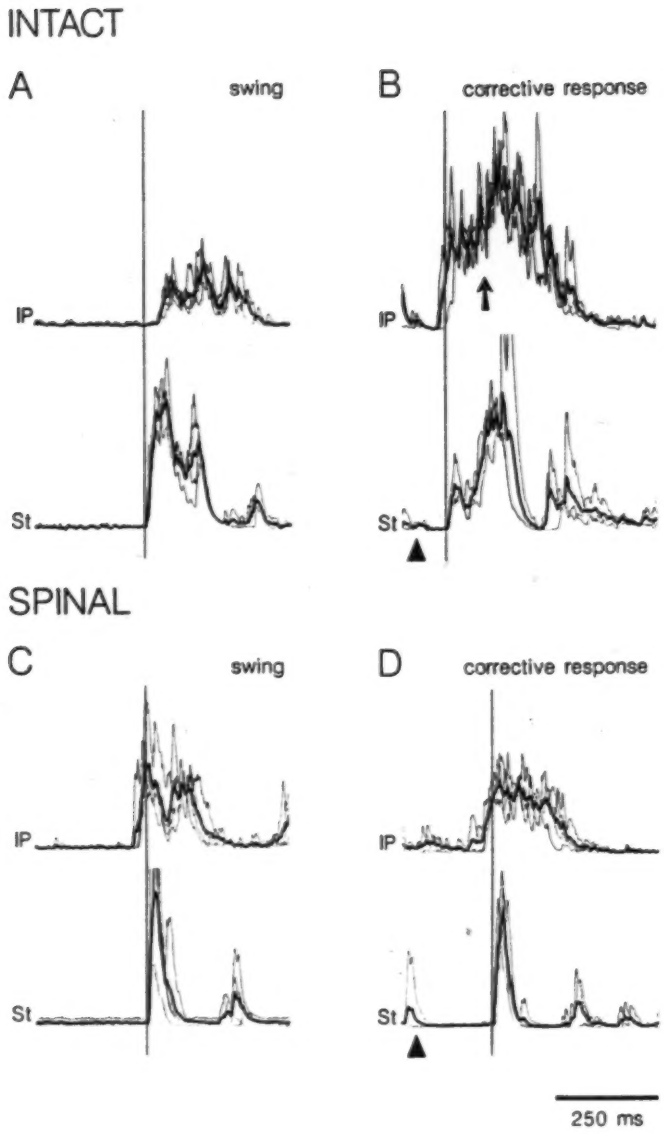

Figure 2.7. Rectified and averaged EMGs (thick lines) from flexor muscles showing flexor bursts during the swing phase of stepping (A,C) and the corrective response (B,D). The thin lines are individual trials. The vertical lines indicate the onset of ST activity. The arrowheads in B and D indicate the estimated time that the foot entered the hole. A,B - intact animal, C,D - spinal animal. Note the increase in the magnitude of the IP bursts (arrow) during the corrective response in the intact animal, and the longer latency in the onset of flexor burst activity in the spinal animal.

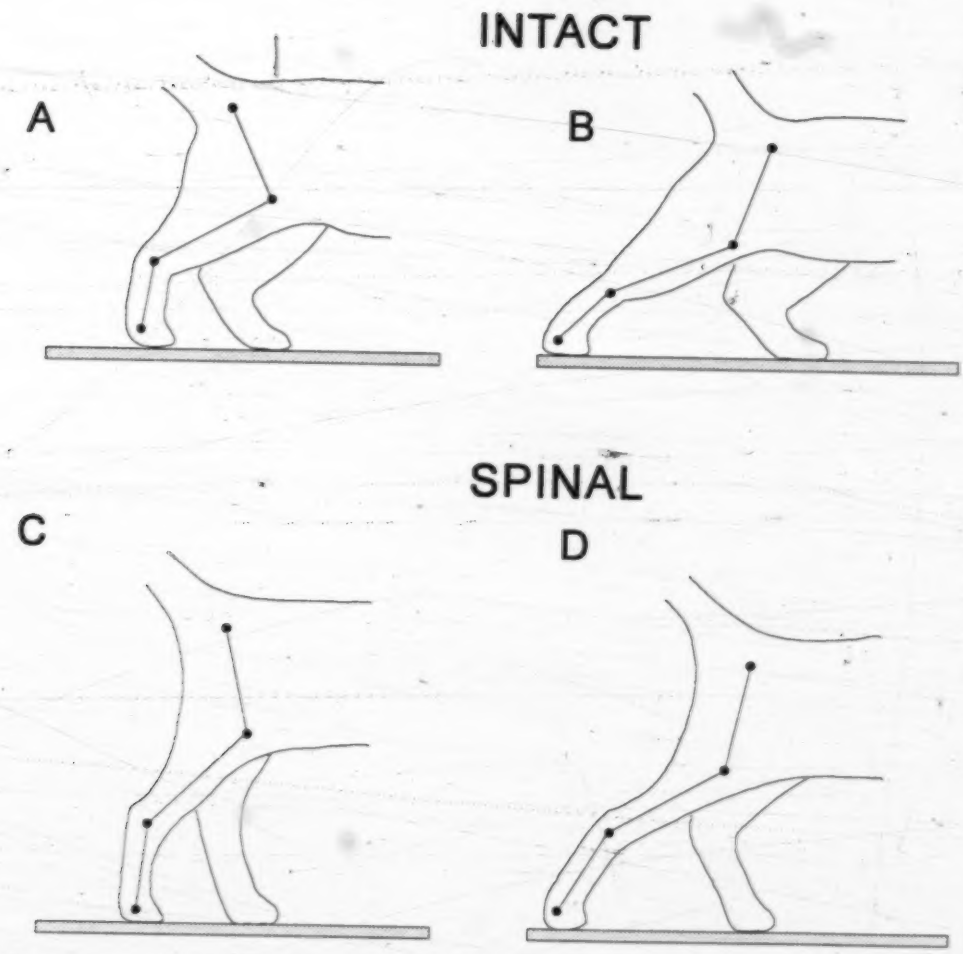

Figure 2.8. Drawings of the positions of the hind legs of an intact cat (A,B) and a chronic spinal cat (C,D) at the end of stance phase when stepping normally on the treadmill (A,C) and at the end of the corrective flexion response after the left leg had been withdrawn from the hole in the treadmill belt (B,D). Note that in both intact and spinal animals the limb contralateral to the hole was extended further than normal.

of the treadmill was kept low. Thus, insufficient data were obtained to analyze responses at higher treadmill speeds.

Corrective responses in the contralateral leg in 3 of 5 chronic spinal cats were generally similar to those in intact animals, i.e. the stance phase of this leg was prolonged to support the hindquarters when the ipsilateral leg entered the hole (Fig. 2.8). In most trials the additional extension of the contralateral leg was sufficient to support the animal's weight during the initial period that the ipsilateral foot was being removed from the hole. In the event that the ipsilateral leg failed to gain support at the end of the flexion response and re-entered the hole, the contralateral leg reached maximum extension and swing was initiated, thus leading to a collapse of the hindquarters. In a few trials in all three of these spinal animals, the swing phase of the contralateral leg began before the initiation of ipsilateral flexion out of the hole. When this occurred, the corrective flexion response was delayed until the swing phase of the contralateral leg was completed. This delayed onset of flexor activity is presumably due to mutual inhibitory coupling between the flexor burst generating systems of the two legs (Grillner 1981; Orsal et al. 1990). In the other two spinal animals the contralateral leg always initiated its swing phase while the ipsilateral leg was in the hole. Thus the flexion of both legs occurred almost synchronously. The hindquarters of these two animals had to be supported by the experimenter when the foot entered the hole.

In all intact animals, and in the three spinal animals that maintained contralateral support during the corrective response, EMG recordings from extensor muscles in the contralateral leg (the right leg) showed an increase in the intensity of extensor activity approximately 70ms after the left paw entered the hole (Fig. 2.9). This latency was similar in both spinal and intact animals, but the magnitude of the increase was usually larger in intact animals. The higher level of activity was maintained for the duration of support.

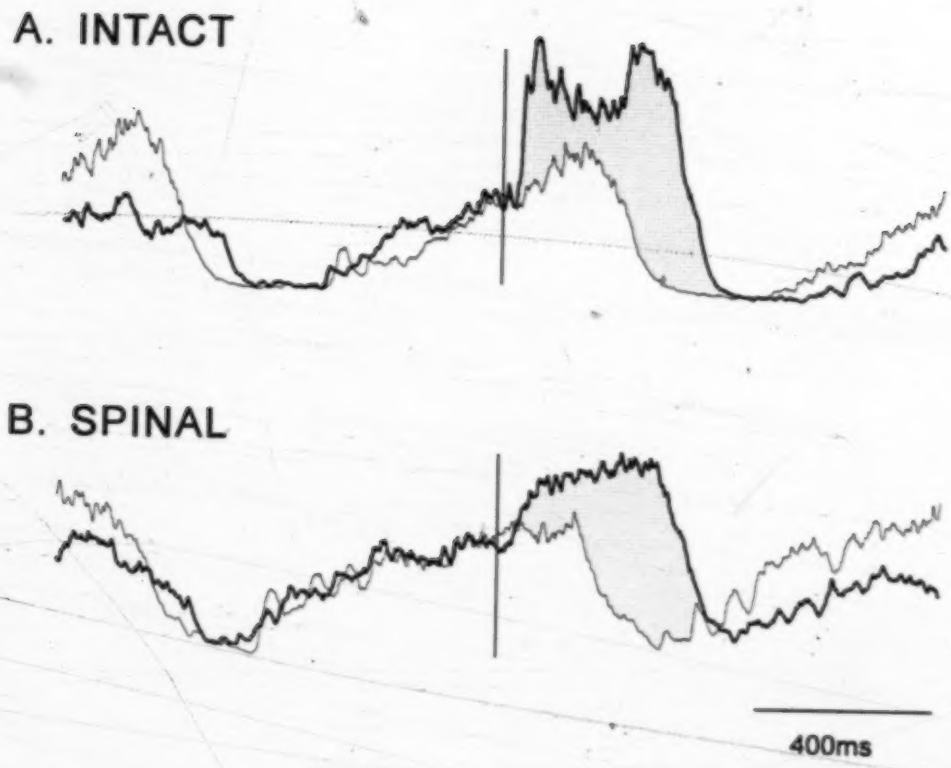

Figure 2.9. Rectified and averaged EMGs from the VL muscle of the contralateral leg in an intact (A) and a chronic spinal (B) cat during normal stepping (thin traces, n=5) and at the time the left leg entered a hole in the treadmill (thick traces, n=5). The vertical lines indicate the times the left paw passed through the light fence. The shaded areas illustrate the increase in the magnitude of the EMGs commencing soon after the left leg entered the hole and the increase in the duration of the EMG activity.

2.4. Discussion.

In an earlier study the corrective response of a hind leg to the unexpected loss of ground support was examined in normal cats walking on a surface containing a hidden trap door (Gorassini et al. 1994). One of the issues arising from that study was whether the response was organized at a spinal level or if supraspinal mechanisms were involved in its initiation and regulation. In this experiment this issue was addressed by comparing the reactions to loss of hind leg support in intact and chronic spinal cats walking bipedally on a treadmill with a hole in the belt. Before discussing this comparison it is important to note that the responses evoked in intact cats stepping bipedally on a treadmill were similar to those occurring during normal quadrupedal walking. This was characterized by a strong flexion of the leg commencing 70 to 150ms after the foot entered the hole and, at low speeds, a marked prolongation of the stance phase of the contralateral leg to support the hindquarters. The flexion movements of the leg were exaggerated compared to these movements during swing. The pattern of muscle activity associated with the kinematics of the corrective response was similar during bipedal and quadrupedal walking. In particular, activity in leg extensors was abruptly terminated soon after the paw entered the hole and, at approximately the same time, bursts of activity were evoked in flexor muscles.

One of the main findings of the present study was that the reaction to a loss of ground support in chronic spinal cats was not the same as that in intact animals. The most obvious differences were that the latency of onset of the corrective flexion movement was variable and significantly longer in the spinal cats (by about 130ms on average: Fig. 2.2), and that an exaggerated flexion movement of the leg did not occur in the spinal cats as compared to the intact animals (Fig. 2.3). Corresponding to these kinematic observations, the latency of onset of flexor EMG activity in spinal cats was delayed significantly compared to that recorded in intact animals (Figs. 2.5B and 2.7) and in spinal animals there was little increase in the magnitude of flexor burst activity relative to the magnitude of swing-phase flexor bursts. These observations clearly demonstrate that supraspinal mechanisms influence the production of the corrective response in intact animals.

The issue now is whether the corrective response in intact animals is generated by a distinct motor program organized at the supraspinal level or whether supraspinal pathways modulate the spinal system that produces the flexion response in spinal animals. The first point to be made is that the spinal system generating the flexor bursts in spinal animals appears to be the same as the system that produces the swing-phase flexor activity during walking. This follows from the fact that the pattern of flexor activity is similar in the two situations (Fig. 2.7C,D). The main difference observed was that the duration of the IP bursts were longer during the corrective response. This may be due to the differences in the leg position at the beginning of the corrective response and at the beginning of stance [in spinal cats it has been reported that the duration and intensity of flexor bursts is dependent on leg position (Pearson and Rossignol 1991)]. The next point is that there are several similarities in the pattern of flexor activity during the corrective response in intact and spinal cats: 1) ST often generated a second weaker burst at the time that extensor activity resumed, 2) the onset of ST activity was delayed slightly compared to the onset of IP activity, and 3) the duration of the IP bursts was prolonged relative to the duration of swing-phase flexor activity. These similarities indicate that the same system is used to produce the corrective response in both groups of animals. Since this is probably the same system generating swing-phase flexor bursts in spinal animals (see above), it follows that the corrective response in intact animals also involves activation of the swing-phase flexor burst generating system. The question now arises as to how this system in intact animals is modulated by supraspinal input. Before considering this, the mechanisms regulating the initiation of flexor activity during stepping and during the corrective response in chronic spinal cats should be discussed.

Two factors are considered to be important in regulating the transition from stance to swing during stepping in spinal cats. The first is extension of the hip (Grillner and Rossignol 1978) and the second is unloading of extensor muscles (Duysens and Pearson 1980; Conway et al. 1987; Pearson et al. 1992). During the corrective flexion response in the spinal animals it was observed that upon entering the hole the limb swung back through the hole until the hip was extended to an angle similar to that observed at the end of stance phase, just prior to the onset of normal swing phase. This observation

suggests the possibility that afferent feedback signalling extension of the limb is contributing to the onset of the corrective reaction to the absence of ground support, an issue that is further addressed in **Chapter 4**.

The other likely cause of the re-initiation of flexor burst activity is the absence of any loading of leg extensor muscles. In decerebrate and spinal animals loading of ankle extensor muscles inhibits the generation of flexor bursts and prolongs the duration of extensor activity (Duysens & Pearson 1980; Conway et al. 1987; Pearson et al. 1992). Thus the complete absence of loading of extensors when the foot enters the hole would be expected to lead to a reduction in the interval between flexor bursts, exactly as observed (Figs. 2.5 and 2.7). The afferents responsible for preventing the initiation of flexor activity when extensor muscles are loaded are most likely the group Ib afferents from the tendon organs (Conway et al. 1987; Pearson et al. 1992; Pearson and Collins 1993). Thus, it is proposed that the flexion response in spinal animals consists of a much earlier onset of swing phase flexor activity because extensor activity generated as the foot entered the hole was not maintained by any input from group Ib afferents (Fig. 2.10A,B). This postulate is further tested in **Chapter 3**.

While it is proposed that the lack of tendon organ feedback is the primary cause of the early flexor response, other proprioceptors could also contribute. For instance, at the transition between E1 and E2 phases of stance, the contracting extensor muscles lengthen as they yield at ground contact thus increasing Ia afferent activity from the muscle spindles (Prochazka et al. 1989). However, when the limb enters a hole there would be no yield and hence a marked decrease in Ia activity. This would result in less excitation of extensor motoneurons and perhaps a reduction in the level of excitation of the interneuronal network generating extensor bursts. The latter may then facilitate the transition from extensor to flexor activity.

How could the mechanism functioning in spinal animals (Fig. 2.10B) be modified and/or what additional mechanisms must function to account for the shorter latency and enhanced magnitude of the corrective response in intact animals? It is unlikely that the flexor burst generating system is tonically facilitated by supraspinal inputs in intact animals since this would result in enhanced flexor activity during the swing phase of

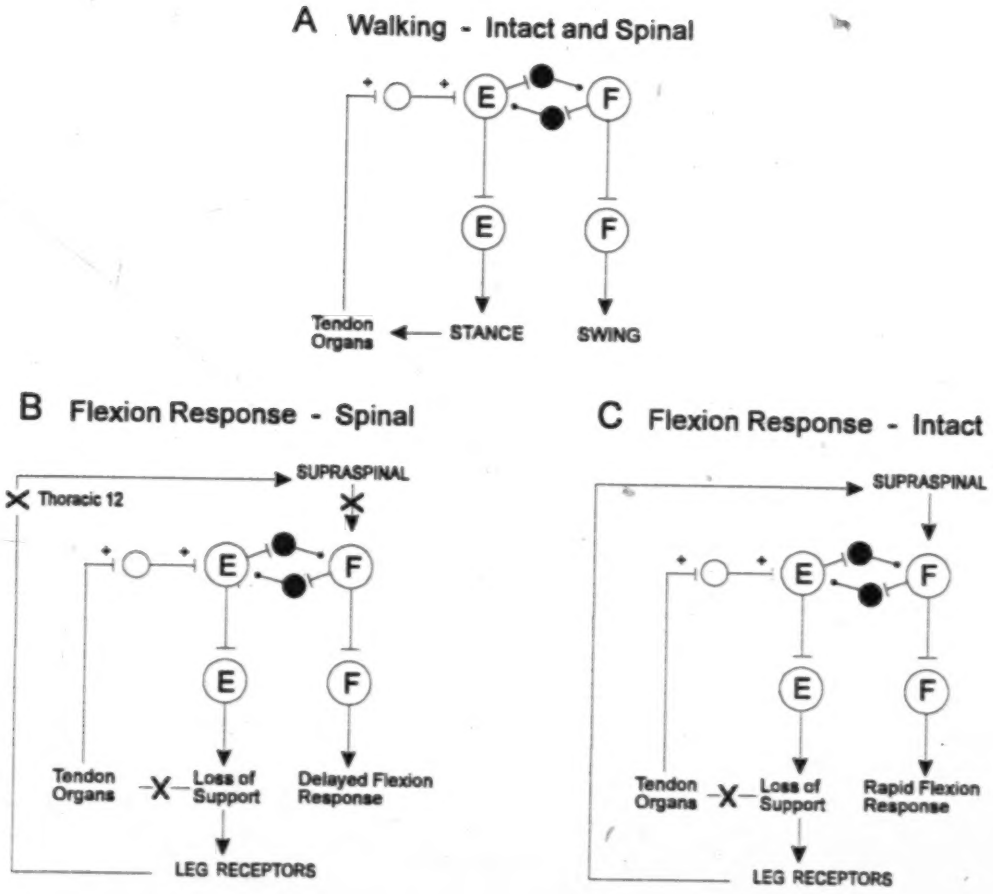

Figure 2.10. Diagrams showing possible schemes for neural pathways involved in walking (A) and in the corrective response to a loss of ground support in spinal (B) and intact (C) animals. During normal walking proprioceptive feedback from the tendon organs (group Ib afferents) ensures that flexor activity is not initiated until the leg is unloaded near the end of the stance phase by inhibiting the flexor half-center via an excitatory pathway to the extensor half-center (the locomotor rhythm generator is represented as mutually inhibiting flexor (F) and extensor (E) half-centers). B) In spinal animals when a leg enters the hole the tendon organs are not activated thus reducing the excitatory input to the extensor half-center which allows flexor burst activity to be re-initiated earlier. C) When a leg enters a hole in intact animals there is also an absence of tendon organ feedback but in this case either the absence of normally occurring stimuli and/or the generation of novel signals activates supraspinal structures to facilitate the flexion response. The crosses in B and C indicate pathways not contributing to the regulation of flexor burst activity.

stepping in intact animals compared to spinal animals. Observations from this experiment and that of Belanger et al. (1990) show that flexor activity during swing is similar in intact and chronic spinal cats. However, it is conceivable that tonic supraspinal signals could facilitate spinal pathways that transmit the novel sensory signals elicited when the foot enters the hole to the network generating flexor bursts. More likely, the novel phasic sensory signals generated when the foot enters the hole (or the absence of an expected sensory signal) could act via supraspinal pathways to trigger a descending command to facilitate flexor burst generation (Fig. 2.10C) (see Appendix I). It should be noted that fairly complex processes could be involved in intact animals since the latency of the onset of flexor EMG activity after the foot entered the hole was between 40 and 80ms. The possibility of phasic supraspinal modulation of spinal pattern generating networks is plausible, since this is known to occur during predictive changes in stepping movements to avoid visually perceived obstacles (Drew 1991). In this case it has been found that the activity of neurons in the motor cortex is facilitated during the enhanced flexion response. It would be interesting to determine whether a similar facilitation occurs in cortical neurons during the corrective response to a foot entering a hole. However, it has been shown that postural reactions in response to a sudden drop of support to one hindlimb are not altered by unilateral ablation of the sensorimotor cortex (Dufossé et al. 1985). Furthermore, preliminary experiments in which specific regions of the motor cortex in the intact cat were reversibly inactivated with lidocaine revealed no alteration in the corrective response to the loss of ground support (Gorassini 1994).

One consistent observation in both intact and spinal animals was an increase in the magnitude of extensor activity in the contralateral leg about 70ms after the ipsilateral leg entered the hole (Fig. 2.9). This increase may have been due to an increase in the load carried by this leg. Other observations demonstrating the dependence of extensor activity on loading conditions are an increase in the magnitude of extensor activity when intact animals walk up an incline (Pierotti et al. 1989), and a reduction in the magnitude of extensor activity during stance in spinal animals when the animal is lifted slightly to reduce loading of the hind legs (personal observations) [this issue is further examined in **Chapter 5**]. In humans afferent feedback is also considered to be involved in the

generation of activity in the soleus muscle during the stance phase of walking (Yang et al. 1991). Alternatively, this contralateral response may have been triggered by sensory signals from the ipsilateral leg. One observation of potential relevance is that in standing animals a drop in the support of one hindlimb results in an increase in extensor activity in the contralateral hindlimb which preceeds the additional loading of the limb (Rushmer et al. 1987).

Another effect of the increased loading of the contralateral leg during the corrective response was a prolongation of the stance phase in all intact animals and in 3 of the 5 spinal animals. The simplest explanation for this phenomenon is that additional loading of extensor muscles acted to reflexly facilitate the circuitry responsible for extensor activity and inhibiting the flexor burst generator. The similar prolongation of the extensor phase in intact and spinal animals, and the qualitatively similar profiles of activity in the VL muscle (Fig. 2.9), indicate that the mechanism regulating the stance phase of the contralateral leg during the corrective response is organized primarily at the spinal level.

In conclusion, this experiment has shown that the speed and vigour of the corrective response to the loss of ground support during walking depends to a large extent on the integrity of supraspinal structures and that descending signals from these structures facilitate the spinal network that normally generates flexor burst activity associated with the swing phase of walking. The identity of the relevant supraspinal structures, and the mechanisms by which they exert the facilatory influence on the locomotor pattern generator, remain to be established (see Appendix I). The data also provides further evidence that afferent feedback can regulate the timing and amplitude of locomotor activity. The characteristic response genenerated during the corrective response to the absence of ground support may prove to be an excellent forum for further investigating the afferents involved the control of locomotion.

2.5. References.

Belanger, M. , Drew, T. , and Rossignol, S. Spinal locomotion: a comparison of the kinematics and the electrophysiological activity in the same animal before and after spinalization. *Acta Biol. Hung.* 39:151-154, 1990.

Berger, W. , Dietz, V. , and Quintern, J. Corrective reactions to stumbling in man: neuronal co-ordination of bilateral leg muscle activity during gait. *J. Physiol.* 357:109-125, 1984.

Conway, B. A. , Hultborn, H. , and Kiehn, O. Proprioceptive input resets central locomotor rhythm in the spinal cat. *Exp. Brain Res.* 68:643-656, 1987.

Dietz, V. , Quintern, J. , and Berger, W. Afferent control of human stance and gait: evidence for blocking of group I afferents during gait. *Exp. Brain Res.* 61:153-163, 1985.

Dietz, V. , Quintern, J. , and Sillem, M. Stumbling reactions in man: significance of proprioceptive and pre-programmed mechanisms. *J. Physiol.* 386:149-163, 1987.

Drew, T. Visuomotor coordination in locomotion. *Curr. Opin. Neurobiol.* 1:652-657, 1991.

Drew, T. and Rossignol, S. Forelimb responses to cutaneous nerve stimulation during locomotion in the cat. *Brain Res.* 329:323-328, 1987.

Dufossé, M. , Macpherson, J. , Massion, J. , and Syberka, E. The postural reaction to the drop of a hindlimb support in the standing cat remains following sensorimotor cortical ablation. *Neuroscience Letters* 55:297-303, 1985.

Duysens, J. D. and Pearson, K. G. Inhibition of flexor burst generation by loading ankle extensor muscles in walking cats. *Brain Res.* 187:321-332, 1980.

Forssberg, H. Stumbling corrective reaction: a phase dependent compensatory reaction during locomotion. *J. Neurophysiol.* 42:936-953, 1979.

Gorassini, M. A. The control and use of proprioceptive information during purposeful movement. Ph.D. thesis. University of Alberta , 1994.

Gorassini, M. A., Prochazka, A. K., Hiebert, G. W., and Gauthier, M. J. A. Corrective responses to loss of ground support during walking I. Intact cats. *J. Neurophysiol.* 71:603-610, 1994.

Grillner, S. Control of locomotion in bipeds, tetrapods and fish. In: *Handbook of Physiology. Sect 1, Vol 2. The Nervous System, Motor Control*, edited by V. B. Brooks. Bethesda: American Physiological Society, 1981, p. 1179-1236.

Grillner, S. and Rossignol, S. On the initiation of the swing phase of locomotion in chronic spinal cats. *Brain Res.* 146:269-277, 1978.

Orsal, D. , Cabelguen, J. -M. , and Perret, C. Interlimb coordination during fictive locomotion in the thalamic cat. *Exp. Brain Res.* 82:536-546, 1990.

Pearson, K. G. , Collins, D. F. Reversal of the influence of group Ib afferents from plantaris on activity in medial gastrocnemius muscle during locomotor activity. *J. Neurophysiol.* 70:1009-1017, 1993.

Pearson, K. G. , Ramirez, J. M. , and Jiang, W. Entrainment of the locomotor rhythm by group Ib afferents from ankle extensor muscles in spinal cats. *Exp. Brain Res.* 90:557-566, 1992.

Pearson, K. G. and Rossignol, S. Fictive motor patterns in chronic spinal cats. *J. Neurophysiol.* 66:1874-1887, 1991.

Pierotti, D. J. , Roy, R. R. , Gregor, R. J. , and Edgerton, V. R. Electromyographic activity of cat hindlimb flexors and extensors during locomotion at varying speeds and inclines. *Brain Res.* 481:57-66, 1989.

Prochazka, A. , Trend, P. , Hulliger, M. , and Vincent, S. Ensemble proprioceptive activity in the cat step cycle: towards a representative look-up chart. *Prog. Brain Res.* 80:61-74, 1989.

Rushmer, D. S. , Macpherson, J. M. , Dunbar, D. C. , Russell, C. J. , and Windus, S. L. Automatic postural responses in the cat: Responses of proximal and distal hindlimb muscles to drop of support from a single hind- or forelimb. *Exp. Brain Res.* 65:527-537, 1987.

Wand, P. , Prochazka, A. , and Sontag, K. M. Neuromuscular responses to gait perturbations in freely moving cats. *Exp. Brain Res.* 38:109-114, 1980.

Yang, J. F. , Stein, R. B. , and James, K. B. Contribution of peripheral afferents to the activation of the soleus muscle during walking in humans. *Exp. Brain Res.* 87:679-687, 1991.

CHAPTER 3.

Suppression of the corrective response to loss of ground support by stimulation of extensor group I afferents.*

3.1. Introduction.

Afferent feedback from peripheral receptors has been shown to modify both the amplitude and the timing of the motor output during walking (Rossignol et al. 1988). One role of afferent feedback is to provide information regarding the state of the animal in relation to the external environment. This information allows the animal automatically to correct for disturbances that occur during walking. For example, stimuli applied to the dorsum of a cat's paw during swing results in increased flexor activity to lift the limb over the source of the stimuli (Forssberg 1979). In Chapter 2 a corrective reaction that occurs when one limb encounters a hole in the supporting surface was characterized. Briefly, upon entering the hole the extensor activity which would normally accompany the onset of stance is abruptly terminated and replaced by a flexor burst that acts to lift the limb out of the hole. The limb is then placed at a new location in front of the hole.

In Chapter 2, it was postulated that one factor in initiating the corrective response to the loss of ground support was the absence of activity in force sensitive Ib afferents which would otherwise reinforce and maintain activity in the interneuronal network generating extensor bursts. Another contributing factor could be the absence of feedback from the group Ia afferents that would normally be generated as the extensor muscles yield during the initial part of stance (Prochazka et al. 1989). Thus, the following experiments investigate the hypothesis that if extensor group I afferents were artificially

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* A version of this chapter has been published by G.W. Hiebert, P.J. Whelan, A. Prochazka and K.G. Pearson in the Journal of Neurophysiology, 73:416-420, 1995. I was involved in all of the experiments, performed most of the data analysis and prepared all figures. I also wrote the initial version of the manuscript.

activated as the limb entered the hole then extensor activity would be maintained and the early transition from extensor to flexor activity would not occur. In order to test this hypothesis various hindleg extensor nerves were electrically stimulated during walking in decerebrate cats. The data show that the flexion response out of a hole can be suppressed by artificially supplying group I afferent feedback from extensor muscles.

3.2. Methods.

Seven intact adult cats (weight 2.5 - 3.5 kg) were used in this investigation. All procedures were carried out with approval from the University of Alberta Health Sciences Animal Welfare Committee.

3.2.1. Experimental preparation.

Each cat was anesthetized with halothane and the trachea was cannulated for continued administration of the anesthetic. One of the carotid arteries was ligated and a cannula to monitor the blood pressure was placed in the other. A cannula was inserted in one jugular vein for the administration of fluids and drugs. Bipolar electromyogram (EMG) electrodes were sutured into various flexor and extensor muscles of the hind legs: medial gastrocnemius (MG), vastus lateralis (VL), vastus medialis (VM), iliopsoas (IP), semitendinosus (ST) and tibialis anterior (TA).

Various extensor nerves of the left hind leg (lateral gastrocnemius-soleus [LG/S], and/or plantaris [Pl] and/or vastus lateralis/intermedius [VL/VI]) were cut close to the insertion into the muscles and freed from the connective tissue for a length of about 1cm. Each nerve was then inserted into a small latex stimulating cuff electrode. The leads from the EMG and stimulating electrodes were run under the skin to a multiterminal connector located near the middle of the animal's back.

A partial laminectomy of the L4 vertebrae was performed and two stainless steel electrodes were placed on the dorsal surface of the spinal cord to monitor the strength of stimuli delivered to the extensor nerves. In one animal a recording nerve cuff, made of silastic tubing with deinsulated wire sutured to the internal surface, was placed around

the sciatic nerve to confirm that the cord dorsum potentials were an accurate indication of the threshold stimulating intensity. The stimulating thresholds for the nerves were determined by monitoring the smallest observable evoked potential at the sciatic or cord dorsum recording site. The strength of the applied stimulus was then measured as a multiple of the threshold (xT) stimulus.

After the dissection, the animal was placed in a stereotaxic head holder and positioned above a treadmill. The animal was then decerebrated by transecting the brainstem at a 50° angle from the anterior edge of the superior colliculus using a stereotaxically guided spatula. Following decerebration the anesthetic was discontinued.

3.2.2. Initiation of locomotion and nerve stimulation.

About 1 hour after decerebration 4 of the 7 animals walked spontaneously on the moving treadmill belt (speed 0.15 - 0.35 m/sec). The duration of spontaneous locomotion varied from animal to animal (0.5 - 5 hours). When there was no longer reliable spontaneous stepping, locomotion was induced by electrical stimulation (15 Hz, 0.6 ms duration, 30-100 μA) of the mesencephalic locomotor region (co-ordinates P2,L4,H6, Shik et al. 1966). Lateral balance was maintained by holding the tail.

The treadmill belt had a hole (10x30cm) cut into the left side into which the left leg would periodically step. Foot entry into the hole was monitored with a light fence, as described in Chapter 2. The light fence signal was used to trigger the stimulation of various extensor nerves at strengths which selectively activated group I afferents (1.6-2 xT, 200-1000 ms duration, 150-200 Hz).

3.2.3. Data recording and analysis.

The recording of data and subsequent analysis is similar to that described in Chapter 2. Briefly, surface markers placed on the skin to derive relative joint positions and limb movements were monitored using a video camera (60 frames/s). The filtered and amplified EMGs, cord dorsum potentials, stimulus signal, and light fence signal were recorded on VHS tape.

A hard copy of the data was displayed on a chart recorder and selected sections data subsequently stored onto a computer disc using Axotape software (Axon Instruments). Custom written software was used to display the data and establish the timing of the EMG events relative to the activation of the light fence and stimulus trains applied to the nerves. The mean latency and standard deviation for the onset of the flexor bursts were calculated.

A frame-by-frame analysis of the video recordings was used to determine the time of onset (to within 16 ms) of the first visible flexion movement at the hip, knee or ankle following the entrance of the limb into the hole. Limb position in individual video frames was analyzed using Video-Blaster software (Creative Labs, Inc.) and custom written programs.

3.3. Results.

Following decerebration 4 of the 7 animals produced a spontaneous walking rhythm. Walking was further facilitated by MLR stimulation. Two of the 7 required MLR stimulation at all times to produce walking. One animal failed to walk after decerebration.

3.3.1. Corrective flexion response in decerebrate animals.

Corrective flexion movements in response to one hind leg entering a hole in the treadmill surface were elicited in all six of the animals that were capable of walking following decerebration. The mean latency from the time the limb entered the hole until a visible flexion movement was initiated at the hip, knee, or ankle was 190 ± 16 ms after the foot passed below the plane of the treadmill belt (Fig. 3.1A). The kinematic response to the hole in the treadmill surface was similar in a number of respects to that observed in spinal animals (see Chapter 2) in that: 1) the limb was strongly extended upon entering the hole, 2) the latency of the corrective flexor activity was slower than that of intact animals, and 3) although a flexion movement was initiated it was not always strong enough to lift the foot clear of the hole and reestablish support. The strength of the

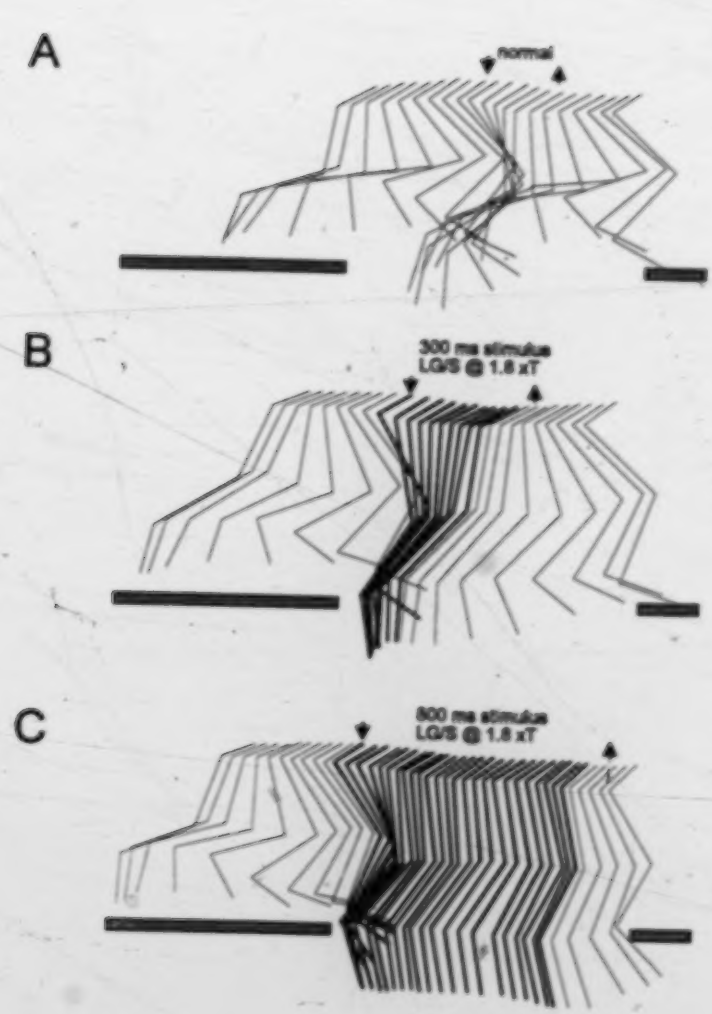

Figure 3.1. Stick figures showing the kinematics of the corrective flexion reaction in response to a hole and the suppression of the flexion response by stimulation of extensor group I afferents in a spontaneously walking decerebrate cat. The arrows indicate the time the limb enters the hole (↓) and the onset of flexion out of the hole (↑). (A) The normal corrective flexion response observed in the decerebrate animal. (B) and (C) The maintained extension of the limb in the hole resulting from the stimulation of extensor group I afferent (thicker lines indicate time that the LG/S nerve was stimulated at 1.8 xT). Note that approximately 70 ms after the termination of a 300 ms stimulus train flexion of the limb was initiated. For clarity, the progression of the traces has been shifted to the right by the corresponding distance moved by the treadmill. The time interval between each trace is 33 ms.

corrective response depended upon the walking pattern present during the perturbation. It was noted, but not quantitatively measured, that a strong flexion response typically occurred during trials where the walking was regular and vigorous. Conversely, during weak locomotion (poor weight support during stance and weak flexion during swing phase) the likelihood of a corrective flexion response being initiated was reduced.

The EMG recordings showed that the motor pattern during the corrective response in decerebrate animals was also similar to that occurring in spinal animals. Extensor activity that typically began just before the foot entered the hole was abruptly terminated as the limb entered the hole. At the same time a burst of activity occurred in flexors to lift the leg out of the hole (Figs. 3.2A and 3.3A). The latency to the onset of flexor activity after entering the hole was 167 ± 37 ms in IP and 180 ± 35 ms in ST. These values are very similar to those recorded in spinal animals as documented in Chapter 2.

3.3.2. Effect of stimulating extensor group I afferents on the corrective response.

In two animals, a flexion movement of the limb entering the hole in the treadmill belt was recorded in 37 out of 42, and 55 out of 65 trials respectively. In the same two animals the flexion response was prevented in 14 out of 14, and 21 out of the 22 trials, by stimulating VL/VI and PI, or LG/S nerves at strengths sufficient to recruit the group I afferents (1.8 xT). The stimulus trains (200-1000 ms) were triggered by disruption of the light fence as the foot entered the hole. Instead of a flexion movement being initiated, there was a maintained extension of the ankle and knee joints for the duration of the applied stimulus. Similar qualitative observations were made in the other four animals.

The lack of a flexion movement was associated with the suppression of a burst of flexor activity that normally occurs soon after the limb enters the hole (Fig. 3.2). Throughout the period of group I stimulation there was maintained low level of activity in the extensor muscles (Figs. 3.2B and 3.3B-F) which corresponded to the leg being held in extension. The extensor activity generated by the stimulus was not limited to those muscles acting at the same joint as the muscle innervated by the stimulated nerve. For

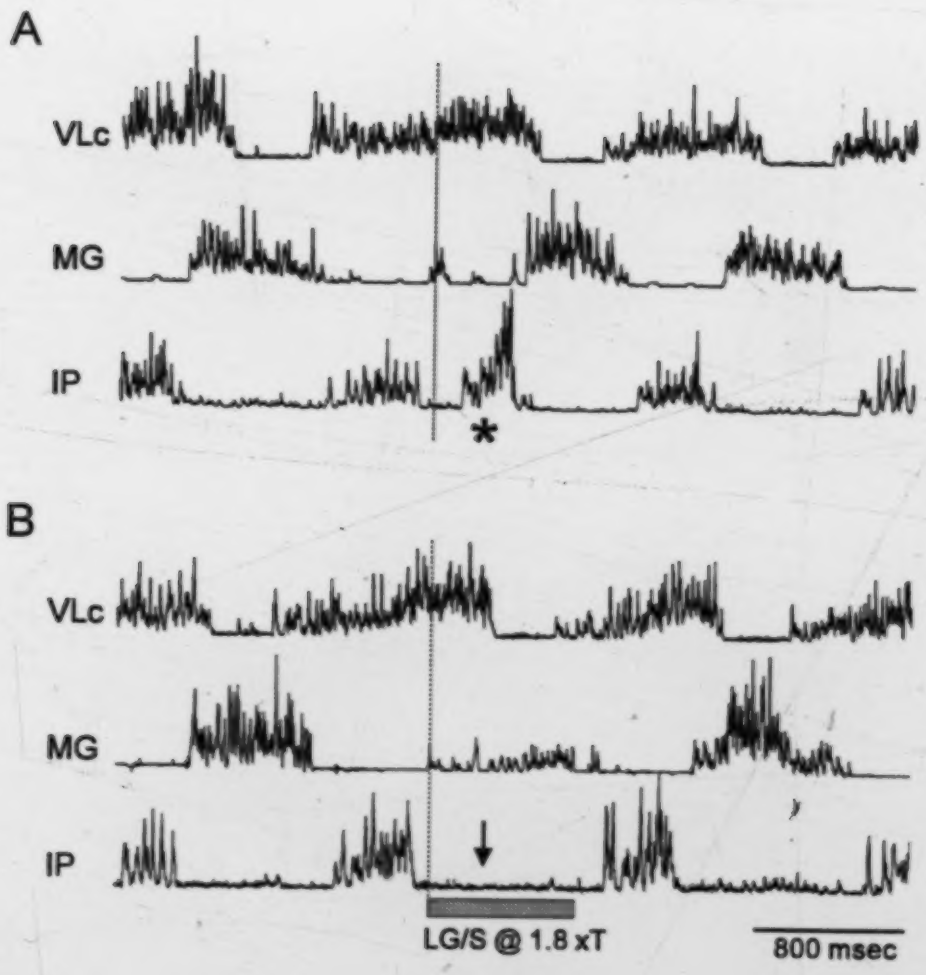

Figure 3.2. A) Filtered and rectified EMG records illustrating the characteristic muscle activity observed when a limb enters a hole in the supportive surface during decerebrate locomotion. Note the burst of activity in IP (•) and the cessation of extensor activity in MG immediately after the limb entered the hole (indicated by the dashed vertical line). B) The motor activity observed when LG/S nerve was stimulated (1.8 xT) as the limb entered the hole. The duration of the stimulus is indicated by the shaded bar. Note the absence of a flexor burst after the limb entered the hole (↑ indicates where flexor activity would usually occur). Flexor activity was resumed upon termination of the stimulus train.

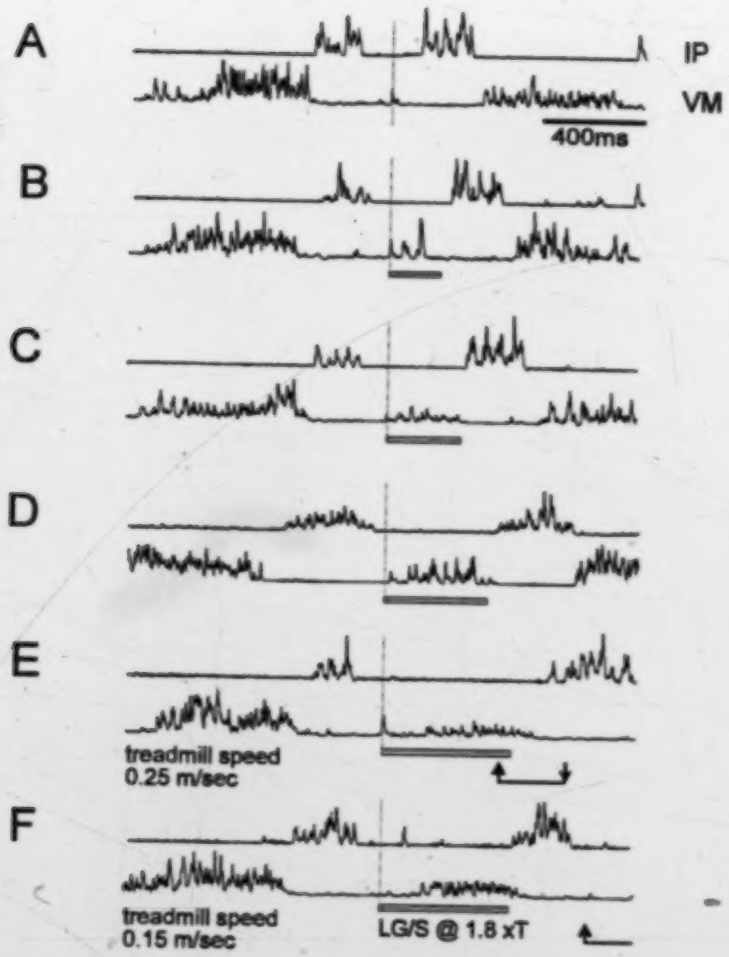

Figure 3.3. Representative filtered and rectified EMG records from VM and IP illustrating the normal corrective response to the absence of ground support (trace A) and the suppression of the corrective response by stimulation of LG/S @ 1.8 xT (shaded bars). The dashed vertical lines indicate the time the limb entered the hole. Note that the onset of flexor activity occurred after the end of 200, 300, or 400ms stimulus (traces B, C, and D respectively). At longer stimulus durations (500 ms, trace E), contralateral support was usually removed before the stimulus was over resulting in a collapse of the hindquarters (the solid line indicates the contralateral swing phase and the arrows indicate toe off and contact). The onset of flexion out of the hole was then no longer correlated to the cessation of the stimulation. If the treadmill speed was decreased (trace F), allowing for a longer period of contralateral support, flexor activity was once again correlated to termination of the stimulus.

example, stimulation of the LG/S nerve resulted in maintained activity in the ankle extensor MG (see Fig. 3.2B), as well as the knee extensor VL (see Fig. 3.3).

Once it had been established that the corrective response could be prevented by stimulating the extensor group I afferents, it was examined whether the timing of the onset of the response could be delayed by stimulus trains that were only slightly longer than the latency of the flexor activity seen during the corrective response. Over a limited range of durations (typically 200 to 400 ms) the corrective flexion movement out of the hole commenced 64-80 ms (4-5 video frames) after the stimulus ended (see Fig. 3.1B). Correspondingly, flexor EMG activity was initiated 70 ± 22 ms after the group I stimulation ended. As the stimulus duration increased, the time from the foot entering the hole to the onset of flexor activity increased (Figs. 3.3B-D).

With longer stimulus trains (>500 ms) the contralateral limb made the transition from stance to swing before the stimulus train ended. When this occurred the flexion of the limb out of the hole was not associated with the termination of the group I stimulus (Fig. 3.3E). Rather the flexion response occurred after contralateral swing had been completed and support returned. By slowing the treadmill to allow a longer period of contralateral support, the association between the onset of flexor activity with stimulus termination was restored (Fig. 3.3F). Conversely, increasing the treadmill speed to reduce the duration of contralateral support decreased the range of stimulus durations over which the onset of flexor activity was associated with the termination of the afferent stimulus volley (data not shown).

3.4. Discussion.

The purpose of this investigation was to examine the mechanism involved in the production of the corrective response that is initiated when the hind leg of a walking cat encounters a hole in the supportive surface. In particular, the hypothesis that the absence of feedback from extensor group I afferents is a factor involved in initiating the corrective response was tested. It is known that at the time of ground contact there is a sharp rise in the discharge rate of both the group Ia and Ib afferents (Prochazka et al.

1989). Studies in rhythmically active acute spinal cats have shown that input from group Ib afferents has an excitatory action on the interneuronal circuitry regulating extensor activity (Conway et al. 1987; Pearson et al. 1992; Pearson and Collins 1993). Thus, the proposal that when a limb enters a hole during walking the absence of feedback from extensor group I afferents shortens the duration of the extensor activity and so promotes an earlier transition from extension to flexion. One prediction of this hypothesis is that electrical stimulation of extensor group I afferents, triggered at the time the foot enters a hole, should suppress the corrective flexion response. The present data confirm this prediction.

Repetitive stimulation of group I afferents from a variety of extensor muscles (LG/S, PL or VL/VI) can produce suppression of the corrective response to the absence of ground support. Coincident with the suppression of the flexor activity was a widespread activation of extensor muscles, ie. there was maintained low level activity in extensor muscles acting at different joints from the muscles innervated by the stimulated nerve. For example, stimulation of LG/S nerve, which innervates ankle extensors, resulted in the moderate activation of VM, a knee extensor (Fig. 3.3). The fact that the extensor activity during group I afferent stimulation was considerably less than that normally occurring during stance phase (see Figs. 3.2 and 3.3) may be explained by the artificial afferent signal from one or two nerves being smaller than the normal signal from many nerves. The maintained activity in the extensors suggests that the group I afferent volley is reinforcing the locomotor centre responsible for the generation of extensor activity. If this is true, then prevention of the corrective flexion response could result from the continued inhibition of the flexor half-centre by the extensor half-centre (see Lundberg 1980, for review of the half-centre hypothesis).

This explanation is consistent with the proposal that a necessary condition for the initiation of swing activity during walking is a decline in the input from extensor group Ib afferents (Duysens and Pearson 1980; Conway et al. 1987). By partially mimicing the activity of the group Ia and Ib afferents that would normally occur had the foot not entered the hole, the duration of extensor burst activity can be precisely regulated according to the duration of the extensor group I afferent volley (Fig. 3.3). Although

these experiments have not demonstrated the relative contribution of input from group Ia and group Ib to the maintenance of extensor activity, previous experiments have demonstrated that input from group Ib afferents are capable of enhancing and prolonging extensor bursts during locomotor activity in reduced spinal preparations (Conway et al. 1987; Pearson and Collins 1993). Also, extensor activity was distributed throughout the limb during LG/S group I stimulation (Figs. 3.1B and 3.3) despite the fact that there are only weak monosynaptic connections between ankle extensor group Ia afferents and quadriceps or hip extensor motoneurones (Edgley et al. 1986). Thus, the assumption that it is primarily the Ib afferents that mediate the suppression of flexor activity when the limb encounters the hole. However, recently group Ia afferents have been shown to have a qualitatively similar excitatory effect on extensor motoneurones as the group Ib afferents (Guertin et al. 1994). The nerve stimulation used in this experiment would certainly have activated the group Ia afferents along with the group Ib afferents. Therefore, it is possible that group Ib and Ia afferents act together, possibly through a set of common interneurons, to generate the observed effect.

Another factor influencing the onset of the corrective flexion response is the state (stance or swing) of the contralateral limb. In intact (Gorassini et al. 1994) and spinal cats (see Chapter 2) it was noted that the corrective flexion response when one limb encountered a hole was not initiated if the contralateral limb was in swing phase. In this investigation it was observed that when the duration of the stimulus trains was increased so they terminated during, or close to, the initiation of the swing phase of the contralateral leg, the onset of the flexion response was further delayed until the time that contralateral swing phase was over and ground support was regained (Fig. 3.3E). One explanation for this observation is that the systems generating flexor bursts in the two hindlegs mutually inhibit each other (Grillner 1981), thus the flexor burst for the corrective response in one leg cannot be initiated during flexor burst activity in the contralateral leg. It has been documented that the intrinsic pattern of hindlimb coordination is most commonly organized so that the step cycles are 180^0 out-of-phase (Orsal et al. 1990). Another possibility is that afferent signals from the contralateral leg

during swing could excite the extensor burst generator and/or inhibit the flexor burst generator.

In conclusion, these experiments have demonstrated that the corrective flexion response to the absence of ground support present in the decerebrate walking cat is similar to that of the spinal animal, and that this response can be suppressed by electrically stimulating group I afferents in extensor nerves. If group Ib afferents contribute to the maintenance of extensor activity, as has been shown during locomotor activity in spinal preparations (Conway et al. 1987; Pearson et al. 1992; Pearson & Collins 1993; Gossard et al. 1994), then an important factor in the initiation of the corrective response to the absence of ground support is the lack of force increase in leg extensor muscles.

3.5. References.

Conway, B. A. , Hultborn, H. , and Kiehn, O. Proprioceptive input resets central locomotor rhythm in the spinal cat. *Exp. Brain Res.* 68:643-656, 1987.

Duysens, J. and Pearson, K. G. Inhibition of flexor burst generation by loading ankle extensor muscles in walking cats. *Brain Res.* 187:321-332, 1980.

Edgley, S. A., Jankowska, E., and McCrea, D. A. The heteronymous monosynaptic actions of triceps surae group Ia afferents on hip and knee extensor motoneurones in the cat. *Exp. Brain Res.* 61:443-446, 1986.

Forssberg, H. Stumbling corrective reaction: A phase-dependant compensatory reaction during locomotion. *J. Neurophysiol.* 42:936-953, 1979.

Guertin, P., Angel, M., Jimenez, J., and McCrea, D. A. Both disynaptic and longer latency internuronal pathways mediate extension enhancement evoked by group I afferents stimulation during fictive locomotion. *Soc.Neurosci.Abst.* 20: 648.8, 1994.

Gorassini, M. A., Prochazka, A. K., Hiebert, G. W., and Gauthier, M. J. A. Corrective responses to loss of ground support during walking I. Intact cats. *J. Neurophysiol.* 71:603-610, 1994.

Gossard, J. -P. , Brownstone, R. M. , Barajon, I. , and Hultborn, H. Transmission in a locomotor-related group Ib pathway from hindlimb extensor muscles in the cat. *Exp. Brain Res.* 98:213-228, 1994.

Grillner, S. Control of locomotion in bipeds, tetrapods, and fish. In: *Handbook of Physiology, sect. 1, The Nervous System, vol. 2, Motor Control, part 2*, edited by V. Brooks, Bethesda MD, p. 1179-1236, 1981.

Lundberg, A. Half-centres revisited. In: *Regulatory Functions of the CNS. Motion and Organizational Principles*, edited by J. Szentagothai, M. Palkovits and J. Jamori. Pergamon Press, p. 155-167, 1980.

Orsal, D. , Cabelguen, J. -M. , and Perret, C. Interlimb coordination during fictive locomotion in the thalamic cat. *Exp. Brain Res.* 82:536-546, 1990.

Pearson, K. G. and Collins, D. F. reversal of the influence of group Ib afferents from plantaris on activity in medial gastrocnemius muscle during locomotor activity. *J. Neurophysiol.* 70:1009-1017, 1993.

Pearson, K. G. , Ramirez, J. M. , and Jiang, W. Entrainment of the locomotor rhythm by group Ib afferents from ankle extensor muscles in spinal cats. *Exp. Brain Res.* 90:557-566, 1992.

Prochazka, A. K. , Trend, P. St. J. , Hulliger, M. , and Vincent, S. Ensemble proprioceptive activity in the cat step cycle: towards a representative look-up chart. In: *Progress in Brain Research. Afferent Control of Posture and Locomotion*, edited by J. H. J. Allum and M. Hulliger. Amsterdam: Elsevier, p. 61-74, 1989.

Rossignol, S. , Lund, J. P. , and Drew, T. The role of sensory inputs in regulating patterns of rhythmical movements in higher vertebrates. In: *Neural control of rhythmical movements in vertebrates*, edited by A. Cohen, S. Rossignol and S. Grillner. New York: John Wiley and Sons, p. 201-283, 1988.

Shik, M. L. , Severin, F. V. , and Orlovsky, G. N. Control of walking and running by means of electrical stimulation of the mid-brain. *Biophysics* 11:756-765, 1966.

CHAPTER 4.

Contribution of hindlimb flexor muscle afferents to the timing of phase transitions in the cat step cycle.*

4.1. Introduction.

It has been demonstrated that the spinal cord alone, in the absence of afferent feedback, is capable of generating the rhythmic alternation of flexor and extensor activity that forms the basis of locomotion (Grillner and Zangger 1984). However, in order for the locomotor output to be adapted to suit the external environment it must be modulated by afferent feedback regarding the state of the moving limbs. Spontaneously stepping decerebrate (Shik et al. 1966) and stepping spinal cats (Forssberg and Grillner 1973) can adjust their locomotor rhythm to match the speed of the treadmill belt. The duration of the stance phase can vary greatly, being long at slow speeds and short at faster speeds. In contrast, the duration of the swing phase remains relatively constant over a large range of speeds (for review see Grillner 1981). These observations suggest that afferent signals play an important role in the timing of the stance phase.

At the present time, there are two proposals regarding the afferent control of the timing of the stance phase to swing phase transition during the locomotor step cycle. Evidence has been presented indicating that force sensitive tendon organ group Ib afferents from extensors are capable of resetting, entraining, and prolonging ongoing extensor activity (Duysens and Pearson 1980; Conway et al. 1987; Pearson et al. 1992; Pearson and Collins 1993). These results indicate that extensor group I afferents are acting on the spinal circuitry regulating extensor activity during locomotion. Thus, one

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proposal is that an unloading of extensor muscles at the end of stance phase is necessary for the swing phase to begin.

The second proposal is that hip extension during stance is a critical signal for the initiation of swing. Grillner and Rossignol (1978) observed in chronic spinal cats stepping on a moving treadmill that by blocking the extension of a limb they could prevent the transition to swing from taking place. If the limb was then allowed to extend, flexion typically occurred when the hip reached an angle of greater than 90 degrees. Sinusoidal movements of the hip have been shown to entrain the fictive locomotor rhythm in spinal and decerebrate cats. During entrainment the onset of flexor bursts occurs near the end of the imposed hip extension (Andersson & Grillner 1983, Kriellaars et al. 1994). The recent work of Kriellaars et al. (1994) demonstrated that afferents from the hip joint capsule were not required for the entrainment and indicated that the afferents from the muscles acting around the hip joint were responsible. The critical muscles and the specific afferents have not been determined.

In the present set of experiments a hypothesis related to the latter of these two proposals was tested, namely that afferent signals generated by the lengthening of the hind leg flexor muscles during stance promote the onset of flexor burst activity associated with swing. Ramp stretches of the hip flexor iliopsoas (IP) and ankle flexors tibialis anterior (TA) and extensor digitorum longus (EDL) were applied at various phases of the step cycle and the resultant changes in EMG amplitude, duration and timing were examined. It was predicted that if stretch-sensitive receptors in these flexor muscles are involved in the initiation of swing then stretch during the stance phase would reduce the duration of extensor activity and promote an earlier onset of flexor activity. The effect of vibrating the flexor muscle and electrically stimulating the nerves to TA and EDL was also examined in an attempt to identify which muscle afferents influence the timing of the stance-to-swing transition.

4.2. Methods.

Eighteen adult cats (2.0 - 4.0 kg) were used in this series of experiments. All procedures were carried out with approval from the University of Alberta Health Sciences Animal Welfare Committee. Each cat was anesthetized with halothane and the trachea was cannulated for continued administration of the anesthetic. One of the carotid arteries was ligated and a cannula to monitor the blood pressure was placed in the other. A cannula was inserted in one jugular vein for the administration of fluids and drugs. After the above initial procedure, one of two different experimental preparations was used. The procedure for each will be described separately.

4.2.1. Decerebrate locomotion with a hind leg immobilized.

The following nerves were transected in the right hind leg of 11 animals: saphenous, sartorius, obturator, hamstrings, superficial peroneal, deep peroneal, sural and distal tibial. The patellar tendon of the right hind leg was cut and a portion of the calcaneum removed to free the triceps surae muscle group. The insertion of IP in the right leg was freed by removing a portion of the lesser trochanter of the femur. A thread was attached to the detached distal tendon of IP, passed between the posterior biceps and semitendinosus (ST) muscles and led from the animal through a cut in the skin of the posterior thigh (Fig. 4.1A). The TA and EDL tendons were also cut and tied to threads to allow later attachment to a muscle puller. Electrodes for recording electromyograms (EMG) were sutured into the muscle bellies of IP, TA, ST, vastus lateralis (VL), and medial or lateral gastrocnemius (MG or LG) of the right hind leg and into IP and/or ST and VL and/or LG of the left hind leg.

In three experiments, the EDL nerve of the freely stepping left hind leg was cut and inserted into a stimulating nerve cuff. A recording cuff was implanted around the sciatic nerve to monitor the strength of stimuli to the EDL nerve. See the Methods in Chapter 3 for a more detailed description.

To fix the back of the animal during the experimental procedure a piece of stainless steel was secured by dental acrylic to the L6 - S1 spineous processes (pins were placed in the base of these processes to attach the acrylic to the spine). The animal was

then held by the back and by a stereotaxic head holder over a motor-driven treadmill, and positioned so that limbs were in contact with the surface of the treadmill. The right hind leg was fixed above the treadmill surface at the knee and ankle. The angle of the hip, knee and ankle joints were held at approximately 90° angles (Fig. 4.1A). This preparation will be referred to as the immobilized or fixed limb (*FL* in the figures) preparation.

Decerebration was performed by transecting the brainstem at a 50° angle from the anterior edge of the superior colliculus and removing the brain. The anesthetic was then discontinued. About 1 hour after decerebration, 8 of the 11 animals began to walk spontaneously with the three free legs on the moving treadmill (speed 0.25 - 0.5 m/s). Rhythmic activity in the right hind leg was coordinated with the stepping movements in the three stepping limbs. Spontaneous locomotion lasted between 1 - 4 hours. When spontaneous locomotion was no longer reliably generated, an attempt was made to induce locomotion by electrical stimulation (15 Hz, 0.6 msec duration, 30-100 mA) of the mesencephalic locomotor region (MLR: co-ordinates P2,L4,H6; Shik et al. 1966).

4.2.2. Decerebrate quadrupedal locomotion.

In this preparation (n=7) the hind limbs were denervated and the patella and Achilles tendon were kept intact. This allowed both hind legs to freely step on the treadmill. This preparation will be referred to as the quadrupedal (*Q* in the figures) preparation. In 5 of the experiments the IP insertion of the right hind leg was freed and attached to a muscle puller (see above). The TA and EDL muscles could not be stretched in this preparation. In 3 animals, a stimulating nerve cuff were implanted around the cut TA nerve and a recording cuff was placed on the sciatic nerve. In 1 of the 3 animals with a TA nerve cuff, the EDL nerve of the contralateral hind leg was similarly implanted with a stimulating nerve cuff and a recording sciatic cuff. This allowed a direct comparison of the two nerves in the same animal.

4.2.3. Activation of the flexor muscle afferents.

Flexor muscle afferents were activated either by stretching (7-8 mm for IP, 5-6 mm for TA and EDL, 50-100ms rise time) or vibrating (150Hz, 50-100μm) individual

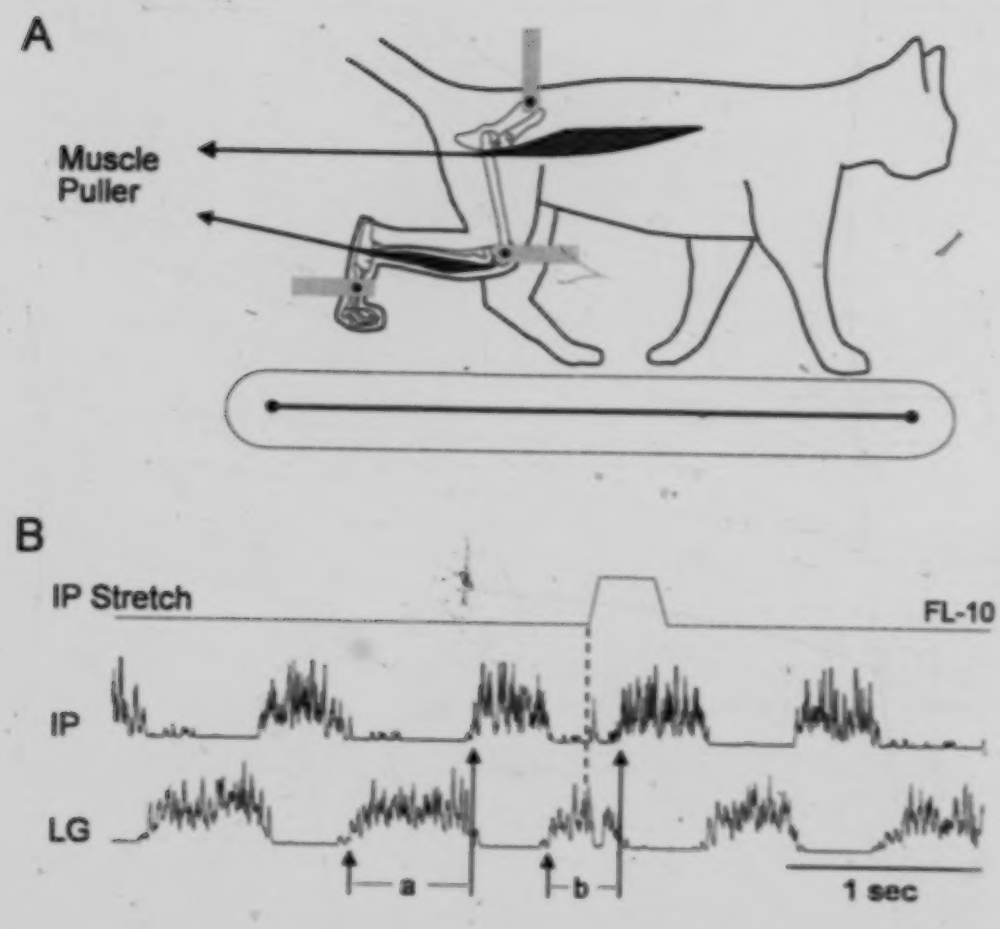

Figure 4.1. Illustration of the experimental set up for stretching the flexor muscles of an immobilized or fixed limb (*FL*) preparation and the EMG events monitored during locomotor activity. A) Decerebrate cats were positioned over a treadmill with the back and the knee and paw of the right leg rigidly mounted to the stereotaxic frame. The insertions of the hip flexor iliopsoas (IP) and the ankle flexors tibialis anterior (TA) and extensor digitorum longus (EDL) were cut and attached to a muscle puller. B) An example of a trial in which IP was stretched: top trace - IP stretch, middle and bottom traces - rectified and filtered EMGs from IP and LG muscles. The duration of extension was calculated as the time between the onset of extensor EMG activity and the onset of the following flexor burst (a). Any change in extensor EMG duration during the stretch of the flexor muscles was calculated as the percent difference of perturbed extensor duration (b) compared to the preceding unperturbed extensor burst (100*(a-b)/a).

muscles or pairs of muscles. The muscles were held at a background tension of 100 grams. Post-mortem dissection in two animals revealed that the lesser trochanter of the femur, which is the insertion point of the IP muscle, moves 10-12 mm as the hip joint is moved from flexion to extension. This is similar to the 9 mm length change of IP during the step cycle calculated by Goslow et al. (1973) for the shortest portion of the IP muscle. The natural change in length of TA and EDL during locomotion is approximately 6 mm (Prochazka et al. 1989). Therefore, the amplitude of stretch imposed during walking was within physiological parameters.

Muscle afferents were also activated using electrical stimulation (150-200Hz, 200-500ms trains) of the nerves to the TA or EDL muscles. The strength of the electrical stimulation applied to the nerves was measured as a multiple of the threshold (xT) strength, from a single shock, required to evoke the smallest observable potential in the sciatic nerve. The onset of the stimuli, electrical or stretch, occurred after a preset delay from the onset of EMG activity in ipsilateral VL or LG to examine the influence during extension, or from the onset of activity in IP to examine the effects during flexion. Custom written programs were used to recognize the onset of EMG bursts, to control the parameters of stretch and vibration, and to trigger the trains of electrical stimulation.

4.2.4. Data analysis.

The data were recorded on VHS tape using a Vetter 4000A PCM recording unit. The set-up allowed us to record 8 channels of data. Due to the limitation of available channels we were not able to simultaneously record all of the implanted EMGs. On occasions some of the implants did not generate a clear EMG and were therefore not used. Furthermore, due to changes in the viability of preparations it was not always possible to investigate all of the flexor muscles or nerves prepared for the experiment.

A hard copy of the data was subsequently displayed on a Gould TA11 chart recorder. Selected sequences were digitized and stored on disc using the Axotape data acquisition system (Axon Instruments). Custom written software capable of reading and displaying the Axotape data files was used to average and display the EMGs and to determine the cycle period and burst durations before and during the applied stimulus.

The duration of extension was calculated as the time between the onset of extensor EMG activity and the onset of the following flexor burst. Any change in extensor EMG duration was calculated as the percent difference of perturbed step cycle compared to the preceding unperturbed step cycle (100*(a-b)/a, see Fig. 4.1B). Statistical analyses using Student's t-test were performed to determine significant differences ($p<0.05$).

4.3. Results.

In 7 of the 11 preparations where one hind leg was immobilized, strong spontaneous stepping movements were evoked in the three unrestrained limbs in response to the moving treadmill belt. MLR stimulation was required to generate a stepping pattern in one animal. Three animals failed to generate any useful stepping sequences either spontaneously or in response to MLR stimulation

Periods of spontaneous locomotion were observed in 5 of the 7 decerebrate quadrupedal walking preparations. Stimulation of the MLR was used in an attempt to prolong locomotor activity after spontaneous stepping had ceased and to generate a locomotor rhythm in the 2 animals that did not demonstrate any spontaneous locomotion. In all quadrupedal preparations, MLR stimulation did not produce any coordinated locomotion.

4.3.1. Flexor muscle stretch during stance phase.

In the immobilized limb preparations an imposed stretch of either IP, TA or EDL midway through the stance phase (100-200 msec after LG or VL onset) caused a reduction in extensor activity. There was a distinct inhibition in the amplitude of the EMG 20-40 ms after the onset of the stretch (Fig. 4.1B). Regardless of whether hip flexor (IP) or ankle flexor (TA, EDL) muscles were stretched, the magnitude of the bursts in the knee (VL) and the ankle (LG) extensor muscles was less than normal during the plateau phase of the stretch. Along with the decrease in extensor EMG amplitude there was also a shortening of the duration of the extensor burst activity as compared to the normal burst duration. Similar results were obtained when the IP muscle was

stretched in the quadrupedal walking preparation. However the decrease in extensor EMG amplitude and burst duration was not as apparent during flexor stretch in the quadrupedal preparation as it was in the immobilized limb preparation.

Data summarizing the reduction in the duration of extensor activity resulting from stretch of the individual flexor muscles are presented in figure 4.2. In all experiments, stretch of each flexor muscle alone produced a significant reduction in the duration of the extensor activity ($p<0.05$). In this and subsequent figures the data from fixed hindlimb preparations are identified with the prefix **FL** and the quadrupedal preparations by the prefix **Q**. In figure 4.2C, when the IP muscle is being stretched, note that the reduction of the extensor duration observed in the quadrupedal preparation, while still significant when compared to the normal extensor burst, was not as pronounced as the reduction observed for a similar stretch in the immobilized limb preparation. On average, stretch of the IP muscle in the 4 immobilized limb preparations reduced the extensor duration by 13%, whereas the reduction during quadrupedal locomotion was 5%.

Associated with the reduction in the duration of extensor bursts was an earlier onset of flexor activity (Figs. 4.1B and 4.3A). Thus, the stretch of the flexor muscles brought about an earlier extensor-to-flexor (stance-to-swing) transition. Although the onset of the flexor activity was earlier than normal, the latency from the onset of the stretch was still large (range 200 - 500ms). This long delay in the onset of the flexor bursts was associated with activity in the contralateral flexors; the ipsilateral flexor burst could not be advanced by flexor muscle stretch to the extent that it overlapped significantly with the contralateral flexor burst (although a small overlap sometimes occurred, ie. Fig. 4.4B). Figure 4.3C illustrates the relationship between the onset of the ipsilateral flexor burst (value Y in Fig. 4.3A) and the onset of the contralateral extensor activity (value X in Fig. 4.3A) following stretch of IP and TA muscles in a fixed limb preparation. The latter value indicates the termination of contralateral flexor activity. The plot in figure 4.3C shows that the onset of the flexor burst following the muscle stretch typically occurred after the onset of the contralateral extension phase and hence after the termination of the contralateral flexor activity. The wide variation of the latency to the onset of ipsilateral flexor activity, apparent in Fig. 4.3C, was due to the variability in the

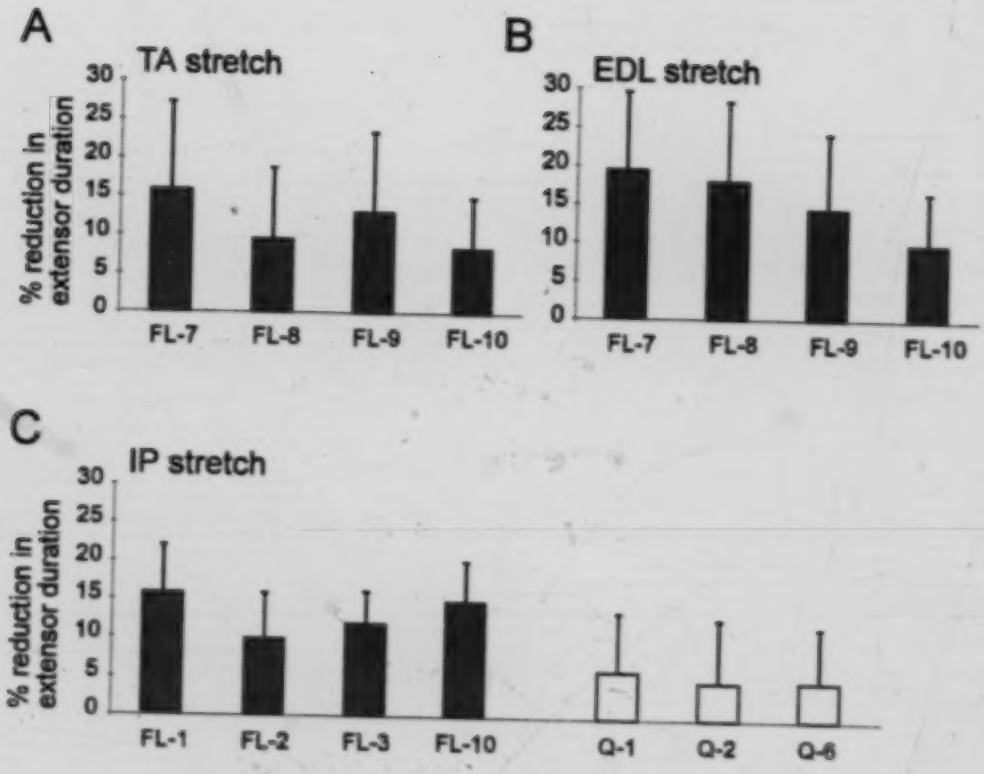

Figure 4.2. Histograms of the mean reduction in extensor burst duration produced by stretch of the individual flexor muscles. Each bar represents data from a single animal (n=15-45 trials for each set of data). The shaded bars indicate results from the fixed limb (*FL*) preparations. The unshaded bars indicate the quadrupedal (*Q*) preparations. The error bars indicate standard deviation. For all data shown the extensor duration was significantly reduced as compared to normal ($p<0.05$). Note that stretch of IP during quadrupedal locomotion resulted in less reduction of the extensor duration than a similar stretch applied to the fixed limb.

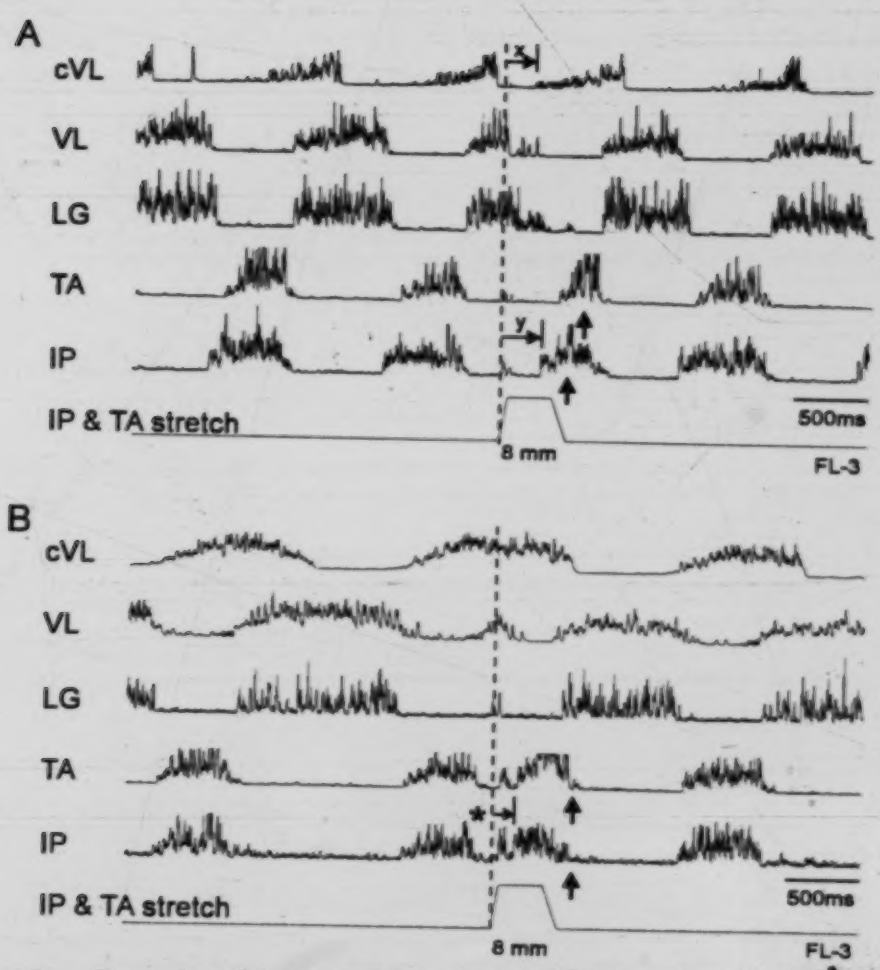

Figure 4.3. Promotion of the onset of flexor burst activity depends on the timing of the muscle stretch relative to the contralateral flexor activity. A and B. Rectified and filtered EMG traces illustrate the reduction in the duration of ipsilateral extensor activity and an early activation of flexor bursts resulting from stretch of IP and TA in a *FL* preparation. The dashed line (- - -) indicates the onset of the stretch and the vertical arrows indicate where the normal onset of activity would have occurred in the flexors IP and TA. A) When the stretch was applied during contralateral flexor activity, ipsilateral flexor bursts (latency value Y) occurred after the onset of the contralateral extensor activity (latency value X). Note that the interval between contralateral extensor bursts (see cVL) was reduced, indicating that there was a reduction in the duration of contralateral flexor activity. B) When the stretch was applied during activity in the contralateral VL, by triggering the stretch earlier and by slowing the treadmill, a rapid onset of ipsilateral flexor activity occurred.

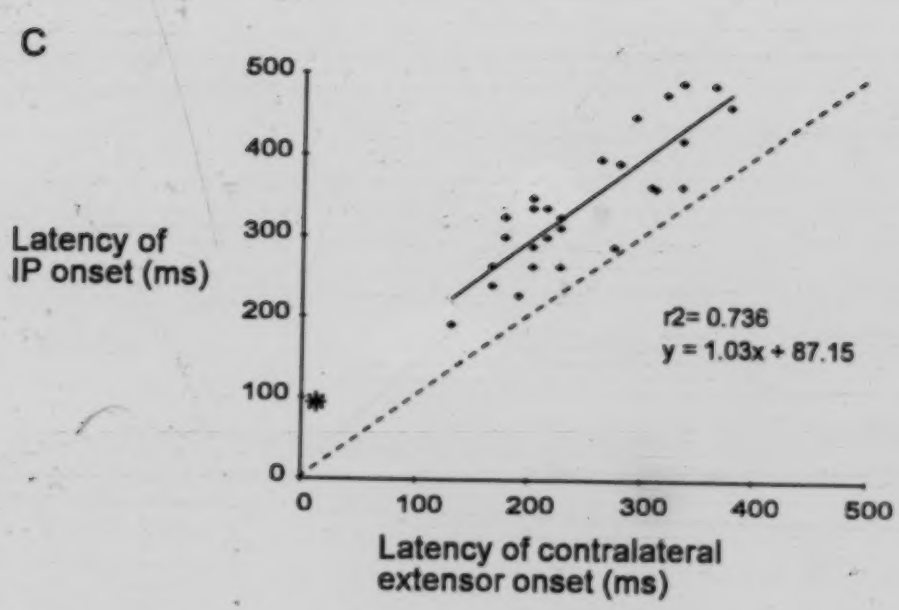

Figure 4.3C. The relationship between the latency of the onset of ipsilateral flexors (value Y) and contralateral extensor bursts (value X) following the flexor stretch as illustrated in Figure 4.3A. The onset of the contralateral extensor burst precedes the onset of the ipsilateral flexor burst. The asterisk represents the latency of IP activation (95 ms ± 10 (SD), n=10 trials) when the muscles were stretched during contralateral extensor activity as illustrated in Figure 4.3B.

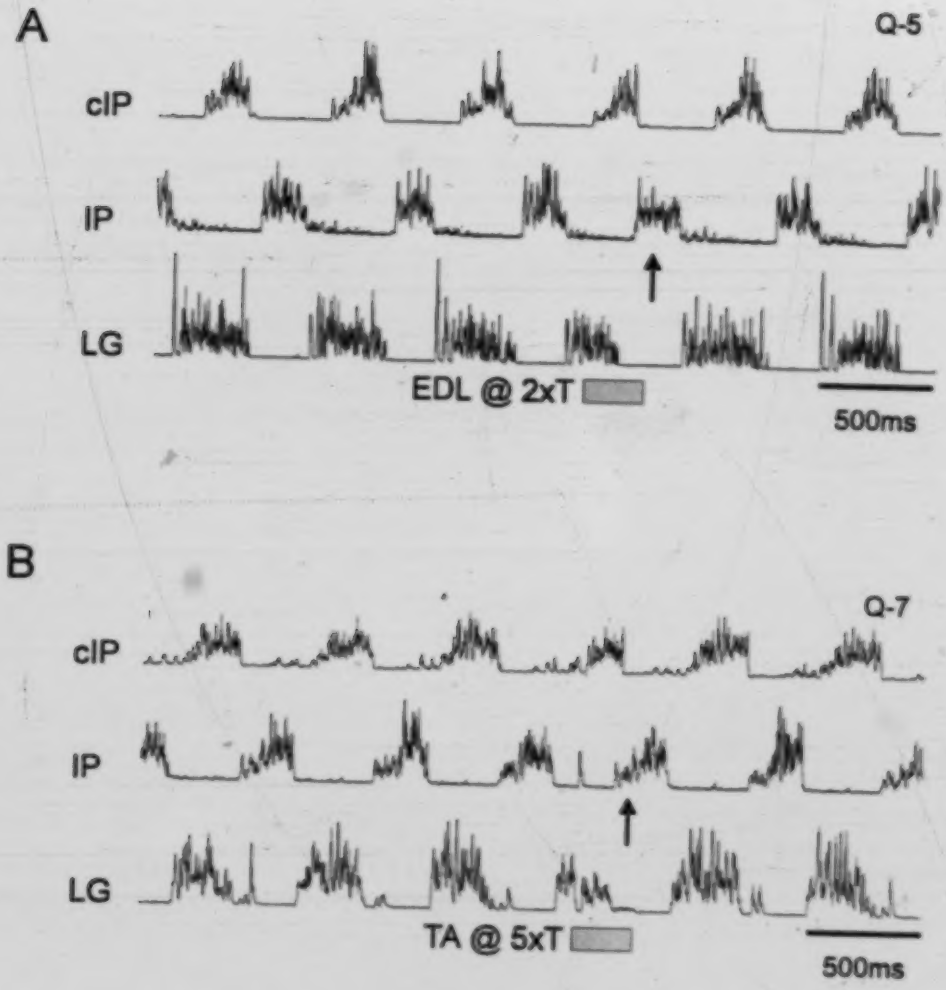

Figure 4.4. Electrical stimulation of EDL nerve (A) and TA nerve (B) resets the locomotor rhythm in a manner similar to that produced by stretching the muscles. The onset of the hip flexor (IP) burst occurred earlier than normal - the arrows indicate where the bursts would have occurred had the rhythm not been modified by the stimulus. A slight reduction in the duration of the contralateral flexor EMG (cIP) also occurred. Note that the stimulus threshold required for such resetting was different for the two nerves. The traces for A and B are taken from two different *Q* preparations.

effect on the contralateral flexor bursts. In some trials there was a marked reduction in the duration of contralateral flexor bursts, as indicated by the reduced interval between contralateral extensor bursts illustrated in Fig. 4.3A. These trials were associated with the shortest latencies to the onset of ipsilateral flexor bursts (clustered around 300 ms in Fig. 4.3C).

In the same experiment as illustrated in Fig. 4.3A, by slowing the treadmill it was possible to generate longer extensor bursts and create a larger period of overlap between the contralateral and ipsilateral extensors. This created the opportunity to time the flexor muscle stretch to occur when the contralateral limb was not in flexion (Fig. 4.3B). In the absence of contralateral flexor activity the mean latency to the onset of ipsilateral flexor burst was reduced to 95 ± 10 (SD) msec. In Fig. 4.3C this value, represented by an asterisk, is plotted against a contralateral extensor latency of zero (we chose this value since the contralateral extensors were active at the time of the stretch). Note that this minimum latency corresponds to the Y-axis intercept of the regression line of the data obtained from trials represented by Fig. 4.3A. (the onset of ipsilateral flexor and contralateral extensor activity when the stretch occurs during contralateral flexor activity).

Thus it appears that at least two opposing factors can influence the timing of the transition from extension to flexion. Stretch-sensitive flexor muscle afferents facilitate the activation of the flexor muscles, while the activity in the contralateral flexor burst generating system prevents their activation.

4.3.2. Electrical stimulation of afferents from ankle flexors during stance phase.

The stretches used to reset the locomotor rhythm would have activated many groups of afferents: group Ia and II afferents from muscle spindles, group Ib afferents from Golgi tendon organs, and group III stretch-sensitive afferents. To gain information as to which of these groups of afferents might contribute to resetting we electrically stimulated the nerves supplying TA and EDL (no attempt was made to place stimulating electrodes on the IP nerves). Based on threshold measurements of identified afferents from the TA muscle (Jack 1978), any effects just above threshold could be attributed to

group Ia afferents, effects beginning in the range 1.3 to 1.5 xT could be attributed to group Ib afferents, and any effects of group II afferents would be seen in the range 2 to 5 xT.

Electrical stimulation of either the TA or EDL nerves (trains of 400 - 600ms at 200Hz) starting approximately midway through an extensor burst reduced the duration of the extensor bursts and promoted an earlier onset of flexor burst activity and contralateral extensor activity (Figs. 4.4 and 4.5). However, there was a clear and consistent difference between TA and EDL nerves in the stimulus strength required to produce this resetting. For the EDL nerve, shortening of the extensor bursts was observed at stimulus strengths of 1.2 xT and the effect was almost maximal at about 1.6 xT (Fig. 4.5A-C). By contrast, TA nerve stimulation only produced shortening of the extensor bursts at strengths greater than 3 xT, and the effect progressively increased as the stimulus strength was increased from 4 to 5 xT (Fig. 4.5D-F). The average magnitude of the reduction in extensor burst duration produced by electrical stimulation of the EDL nerve (2 xT) or the TA nerve (5 xT) was usually in the range of 10 to 20%. Thus in general the effects produced by electrical stimulation of the nerves were comparable to the effects produced by muscle stretch (compare with data shown in Fig. 4.2). The only exception was observed in one quadrupedal walking preparation in which EDL nerve stimulation at low strengths produced exceptionally large (about 40%) decreases in extensor duration (Fig. 4.5C). In the contralateral leg of the same animal, electrical stimulation of the TA nerve had the usual modest effect over the range of 2 to 5 xT (Fig. 4.5F).

4.3.3. Vibration of flexor muscles.

The fact that very low strength electrical stimuli to the EDL nerve promoted the onset of flexor bursts and reduced extensor burst duration suggested that these effects were produced largely by activation of group Ia afferents. To test this possibility the EDL muscle was vibrated in immobilized limb preparations (n=4) to selectively activate the group Ia afferents (Brown et al. 1967). As expected, bursts of vibration (150Hz, 80mm, 600ms) delivered midway through the extensor bursts promoted an earlier onset of ipsilateral flexor activity (Fig. 4.6A).

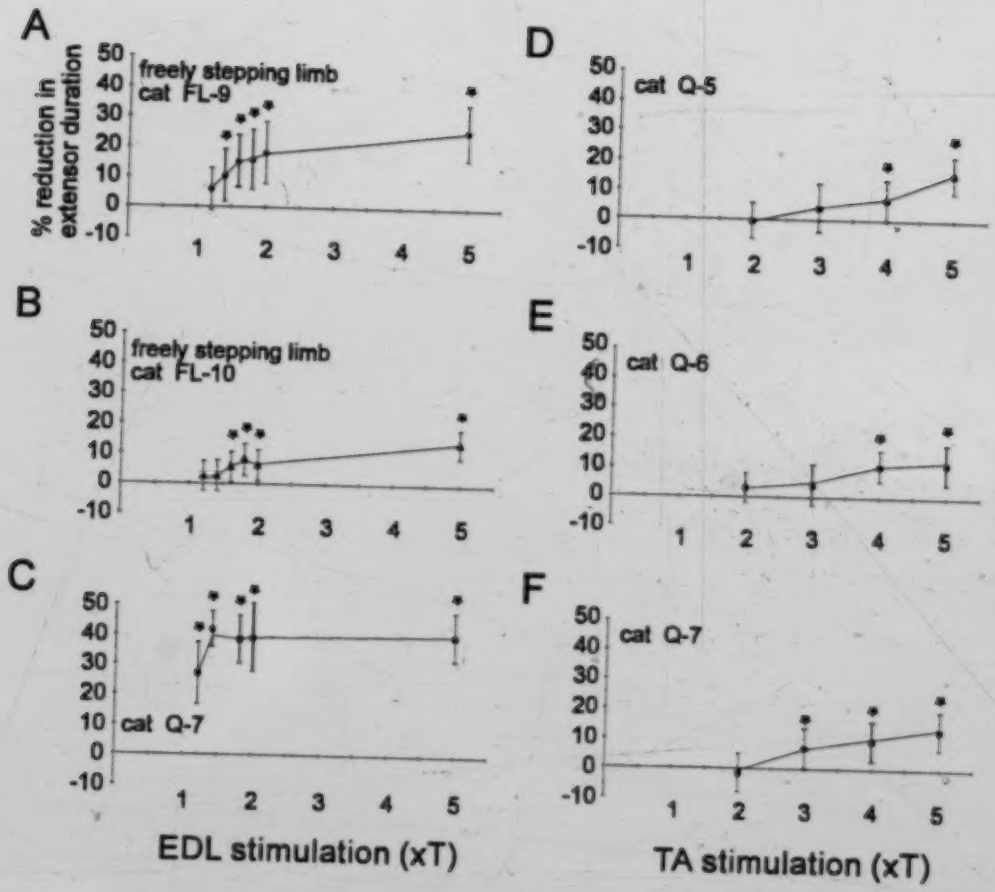

Figure 4.5. Average reduction of the extensor duration by electrical stimulation of the ankle flexor afferents is a function of the strength of the stimulus (n=6-20 trials averaged for each data point). The error bars indicate the standard deviation. A-C. EDL nerve stimulation. D-F. TA nerve stimulation. The data illustrated in A, B, D, and E were obtained in four separate experiments. Data shown in C and F were collected from the same animal. Statistically significant ($p<0.05$) reductions in the extensor durations as compared to normal are indicated by asterisks.

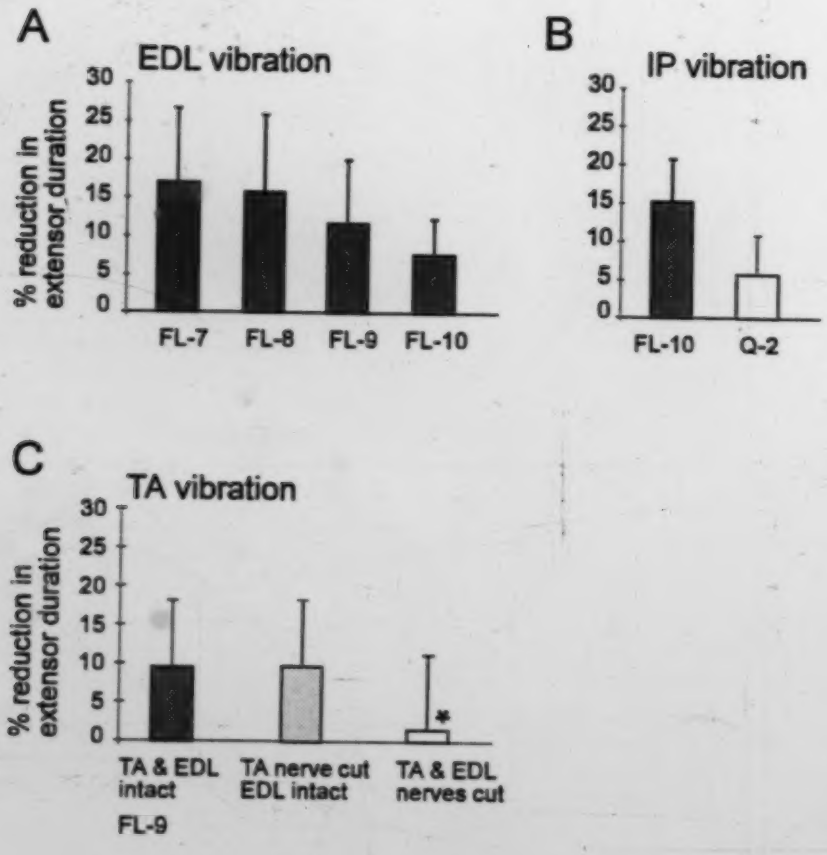

Figure 4.6. Vibration of the flexor muscles reduces the duration of extensor burst activity. A and B. Summary of the mean reduction in extensor duration observed for all experiments in which either EDL or IP was vibrated (n=15-55 trials in each data set). The error bars indicate standard deviation. Note that the reduction produced by IP vibration during quadrupedal locomotion (unshaded bar in B) was less than that observed when a similar vibration was applied in the fixed limb preparation. C) Vibration of TA produced a reduction in extensor activity when both the TA and EDL nerves were intact (dark shaded bar). This effect remained after the TA nerve was cut (light shaded bar). The effect was abolished after cutting both TA and EDL nerves (open bar) (asterisk indicates $p>0.05$).

Similar effects were also observed when the IP muscle was vibrated (Fig. 4.6B). As in the experiments where we stretched IP, the magnitude of the reduction produced by vibration in the immobilized limb preparation was distinctly greater than that observed in the quadrupedal preparation.

An unexpected observation was that vibration of the TA muscle also promoted an earlier onset of flexor activity (Fig. 4.6C). Electrical stimulation of the TA nerve indicated that group I afferents from TA had no influence on the locomotor rhythm. One explanation for this apparent contradiction is that the vibration was spreading to the nearby innervated EDL muscle. This was demonstrated in one preparation by cutting the TA nerve and then vibrating or stretching the denervated TA muscle. Vibration continued to reduce the extensor duration after the nerve was cut (Fig. 4.6C), whereas stretch no longer did (data not shown). Subsequent cutting of the EDL nerve abolished all effects of TA or EDL vibration on the rhythm (Fig. 4.6C). Apart from indicating that the effect of vibration of TA was mediated by afferents in the EDL nerve, this observation showed that the effects of vibration of TA and EDL were not mediated by spread to more proximal muscles.

4.3.4. Entrainment of the locomotor rhythm.

The observation that the locomotor rhythm could be reset by stretching flexor muscles (Fig. 4.3A) suggested that the rhythm could be entrained by repetitively stretching these muscles. Previous studies have shown that rhythmic movements of the hip joint are capable of entraining the fictive locomotor rhythm (Andersson and Grillner 1983; Kriellaars et al. 1994). However, in those experiments there was no phasic afferent feedback except for that resulting from the imposed movement of the hip. It was therefore of interest to examine whether the locomotor rhythm could be entrained in the presence of afferent feedback from the other three freely stepping limbs. The effect of rhythmically stretching the hip and ankle flexor muscles was investigated in 3 of the immobilized limb preparations in which a regular locomotor pattern was generated for long periods of time.

Provided that the frequency of the entraining stretch (a repeating saw-toothed pattern) was no more than 15% greater than the natural locomotor rhythm, the rhythm in the immobilized test limb could be entrained for periods of up to 40s. No entrainment was observed when the frequency of the stretch was set lower than the natural rhythm. An example of entrainment is illustrated in Fig. 4.7 where IP and TA were stretched together. In Fig. 4.7B the phase of onset of the flexor activity within the stretch cycle is plotted against time. Two periods of entrainment are seen to have occurred. Periods of entrainment are indicated by the regions where the phase of flexor onset remained relatively constant. This form of entrainment, where the onset of EMG activity occurs at a preferred phase and then eventually drifts away from the imposed rhythm, has been referred to as relative coordination (Horsmann et al. 1983). The progressive increase in phase, observed when the rhythm was not entrained indicates that the frequency of the imposed saw-tooth stretch was higher than that of the endogenous rhythm. Similar plots were obtained in another preparation for stretch of IP alone, and in this and one other preparation when IP and EDL were stretched together. In none of the preparations was the rhythm sufficiently sustained and regular to test all combinations of flexor muscles, and to compare the strength of entrainment with different combinations.

The timing of the locomotor activity during entrainment was such that the onset of the flexor EMG corresponded to a point near the end of the imposed stretch (see Fig. 4.7A). This indicates that the stretch of the flexor muscles was acting to bring about the initiation of flexor burst activity. Consistent with this was the observation that the duration of the extensor bursts during entrainment was reduced compared to the duration of extensor bursts when the rhythm was not entrained. Thus the reduction in the extensor burst duration created a slightly shorter step cycle period which accounts for the increase in the frequency of the stepping rhythm. These observations are in accord with those of Andersson and Grillner (1983) and Kriellaars et al. (1994).

Rhythmic stretching of the flexor muscles of the immobilized limb also entrained the stepping pattern of the contralateral hind leg (Fig. 4.7B bottom). This occurred despite the fact that the rhythm in the intact contralateral leg (and the rhythm of the forelegs) was strongly coupled to the speed of the moving treadmill belt. The rate of

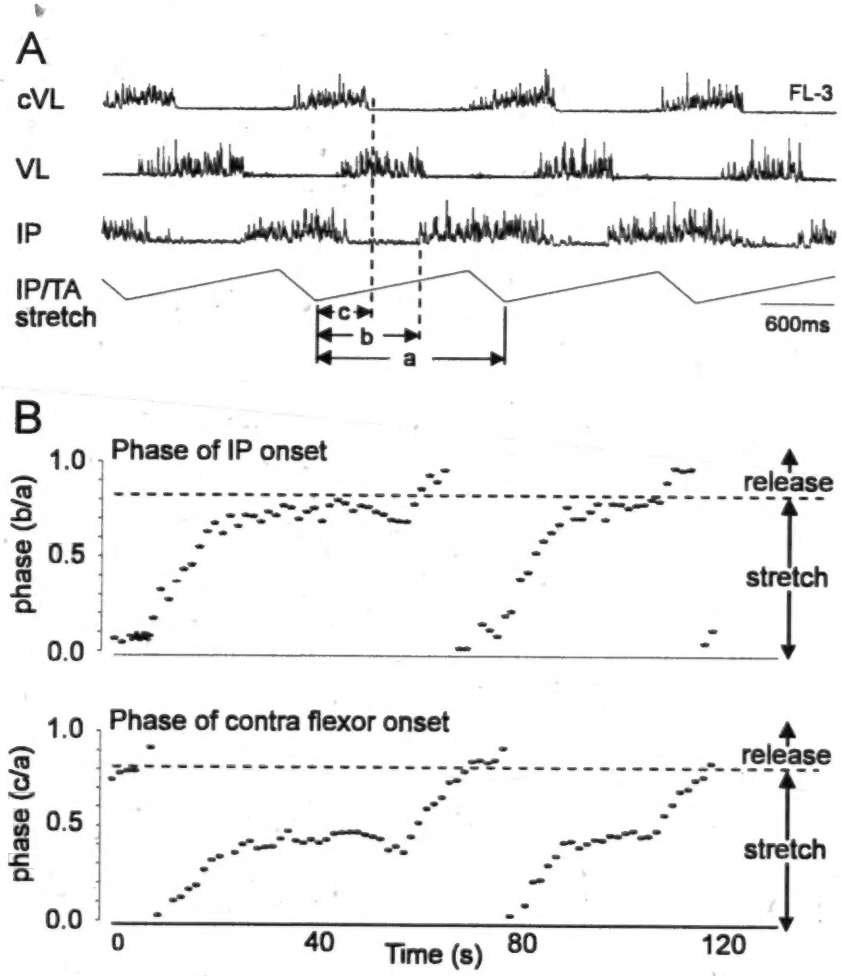

Figure 4.7. Entrainment of locomotor rhythm by saw-toothed stretches of flexor muscles in an *FL* preparation. A) Sample rectified and filtered EMG traces and the imposed saw-toothed stretch (8 mm) of the IP and TA muscles showing the parameters measured to calculate the phase of the onset of the flexor EMG activity: *a* - cycle period of the entraining saw-tooth stretch, *b* - time of onset of the ipsilateral IP activity relative to the onset of the stretch, *c* - time of onset of the contralateral flexor phase (as determined by the end of the contralateral extensor activity) relative to the onset of the stretch. B. Plots showing the phase of the onset of ipsilateral (top) and contralateral (bottom) flexor EMG bursts during the muscle stretch. Note that during entrainment the ipsilateral flexors become active near the end of the imposed stretch. Entrainment of the stepping contralateral limb occurred despite the fact that the timing of stepping of this limb was strongly dependent on the movement of the treadmill.

stepping in the contralateral leg increased during entrainment due primarily to a decrease in the duration of the extensor (stance) phase.

In one immobilized limb preparation entrainment of the locomotor rhythm by repetitively vibrating the IP muscle was possible (Fig. 4.8). When the repetition rate of the bursts of vibration was slightly higher than the natural stepping cadence entrainment could last for substantial periods (up to 30 seconds). In accordance with the observations on the effect of repetitively stretching IP (Fig. 4.7), the timing of the flexor EMG bursts was such that during entrainment activity was initiated near the end of vibration. It should be noted that nearby hip and trunk muscles were not denervated and is therefore possible that some afferents in these surrounding muscles were also excited by the vibration.

4.3.5. Stimulation of flexor muscle nerves during swing phase.

Based upon the current observation that stretch-sensitive afferents from flexor muscles can facilitate the termination of extensor activity and facilitate the onset of flexor activity, we would predict that activation of flexor muscle afferents during flexion would facilitate and prolong flexor activity. However, in recent experiments, it has been reported that stimulation of the nerves to TA, sartorius, and semitendinosus at 5 xT during flexor activity shortened the duration of activity in flexor nerves and evoked activity in ipsilateral extensor nerves (Perreault et al. 1995).

To examine this issue further the IP muscle was stretched (2 quadrupedal preparations), or the TA (1 fixed limb and 3 quadrupedal preparations) or EDL (1 fixed limb and 1 quadrupedal preparation) nerve was electrically stimulated, during flexor activity. Stimulation of the EDL nerve at 1.4 - 2 xT during flexor activity increased the duration of the flexor bursts (measured from the IP burst) in both quadrupedal (Fig. 4.9A) and fixed limb experiments, though not to the 600 ms duration of the stimulus trains. However, with higher levels of stimulation (5 xT) the ipsilateral flexor EMG activity did last for the duration of the applied nerve stimulation (Fig. 4.9B). The prolongation of the flexor burst duration was matched by a larger interval between

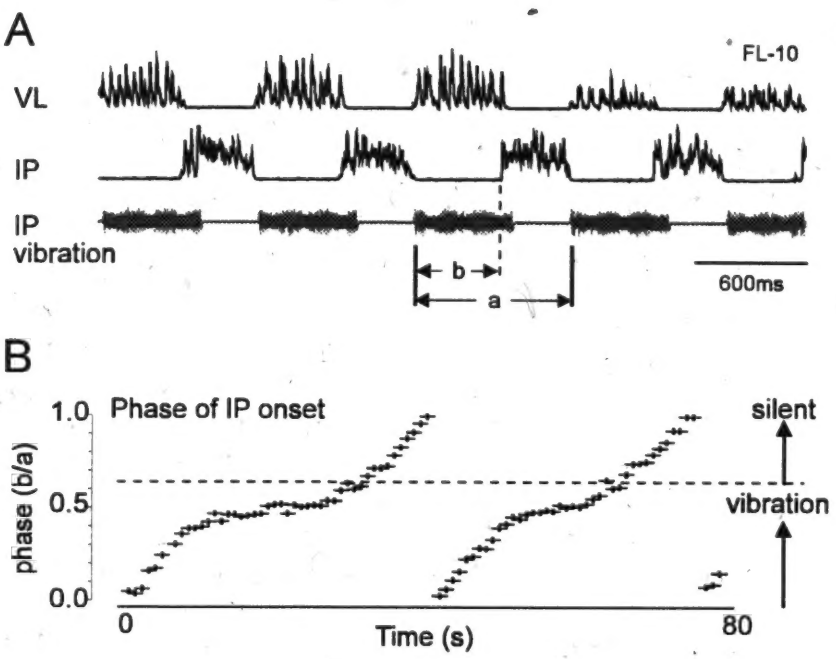

Figure 4.8. Entrainment of the locomotor rhythm by vibration of the IP muscle in an *FL* preparation. A) Sample rectified and filtered EMG traces and the imposed vibrations of the IP muscle showing the parameters measured to calculate the phase of the onset of the flexor EMG activity: *a* - cycle period of the entraining vibration, *b* - time of onset of the ipsilateral IP activity relative to the onset of the vibration. B) Plot showing the phase of the onset of ipsilateral flexor EMG bursts during two periods of entrainment. Note that the onset of flexor EMG activity during entrainment occurred near the end of the vibration. This is similar to the timing of the entrainment when the muscles were stretched in another preparation (see Fig.7).

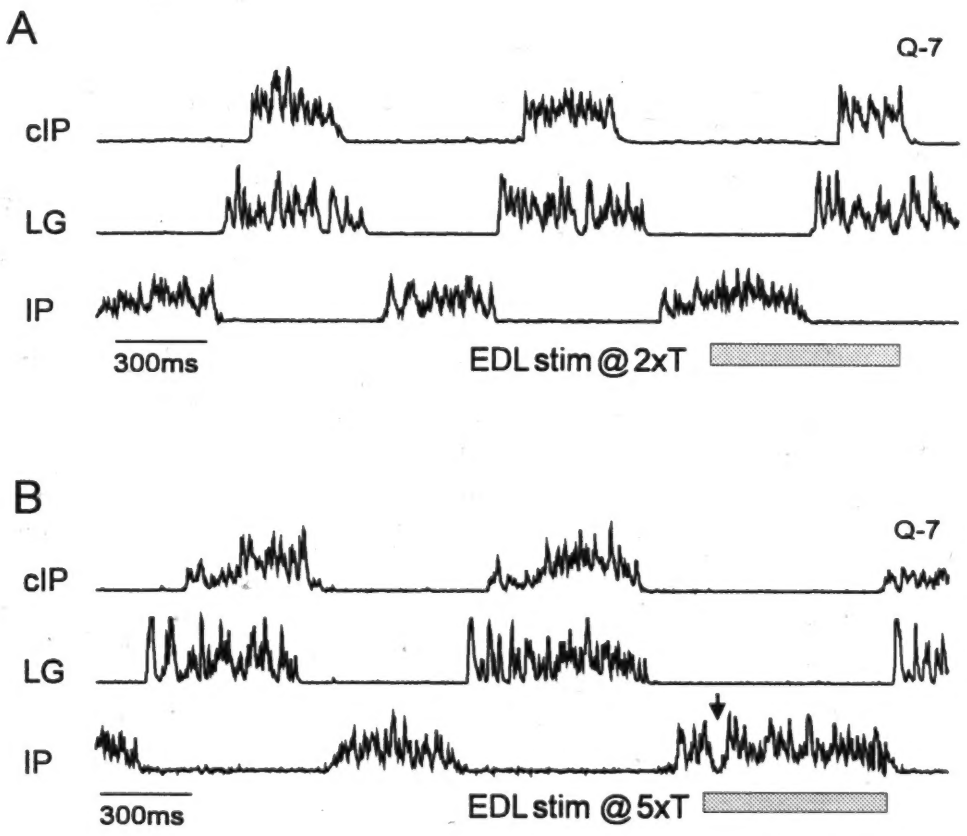

Figure 4.9. Electrical stimulation of the flexor muscle afferents during the flexion phase can prolong the duration of flexor activity. A. Stimulation of EDL at 2xT increased the flexor duration though not for the full duration of the stimulus train. B. Stimulation at 5xT prolonged the duration of flexor activity for the duration of the stimulus. Note the short period of inhibition of the flexor activity just after the onset of the stimulation (arrow).

contralateral flexor bursts, indicating an increase in the contralateral extensor burst durations.

Stretch of IP during flexor activity failed to produce any change in the step cycle rhythm in any preparation: the duration of flexor activity was not increased, nor was there a noticeable increase in the EMG amplitude (data not shown). There was a similar absence of effect in response to electrical stimulation of the TA nerve at 2-5 xT in three of the four experiments. In the fourth preparation there was no effect at 2 xT. However, when TA was stimulated at 5 xT the duration of the flexor activity increased, though not for the duration of the 600 ms stimulation. On average the duration of flexor activity increased by 13% ± 12 (SD) of normal (data not shown).

In none of the preparations was a resetting to extension observed when flexor afferents were stimulated during flexor activity, as reported by Perreault et al. (1995). However, in one preparation we did observe a brief inhibition of IP activity soon after electrically stimulating the EDL nerve at 5 xT but this was always followed by a continuation of flexor activity that led to a prolongation of the flexor burst (Fig. 4.9B).

4.3.6. Stimulation of flexor muscle nerves during the corrective response to the loss of ground support.

In two quadrupedal experiments the treadmill belt was replaced with a belt that had a hole (10x30cm) cut into the left side. This occasionally resulted in the left limb entering the hole and subsequently exhibiting a corrective flexion reaction to the absence of ground support (as described in Chapter 2 and 3). On every second enounter with the hole, flexor muscle afferents were activated by stretch of IP (in the first animal) or by electrical stimulation (2 xT) of EDL nerve (in the second animal).

The onset of activity in IP occurred 205 ± 37 ms and 225 ± 59 after entering the hole during control encounters with hole (Fig. 4.10). This is slightly slower than the latency reported for the corrective flexion response observed in earlier experiments on decerebrate animals (~180 ms, see Chapter 3). If the IP muscle was immediately stretched (0 delay, 8mm, 50 ms ramp) upon the limb entering the hole, activation of IP activity was observed after 166 ± 45 ms. Similarly, stimulation of the EDL nerve resulted

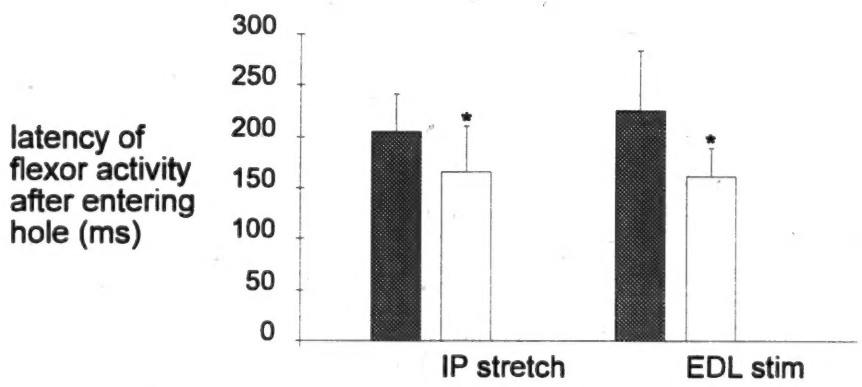

Figure 4.10. Facilitation of the corrective response to the absence of ground support by activating of flexor muscle afferents. Each bar represents the average (n=12) latency of IP activation, from the time the limb entered the hole in the treadmill belt. The unshaded bars illustrate the latency of IP activation when IP was stretched or EDL nerve was stimulated (2xT) as the limb entered the hole, while the shaded bars reprent the control trials. Two separate sets of data are presented, one for each experimental condition. The error bars indicate standard deviation. Stretch of IP or stimulation of EDL resulted in a significant ($p<0.05$, unpaired Student's T-test) reduction in the latency of flexor activation, as indicated by the asterisks.

in the onset of flexor activity after 162 ± 27ms. Both of these values are significantly ($p<0.05$) faster than the undisturbed reaction.

4.4. Discussion.

Previous studies have shown that movements around the hip joint can reset and entrain the fictive locomotor pattern in spinal and decerebrate cats (Andersson & Grillner 1983; Kriellaars et al. 1994). The characteristics of resetting and entrainment have suggested that afferent signals arising from hip muscles during extension could act to trigger the transition from stance to swing in a walking animal (see also Grillner & Rossignol 1978). Since the hip flexor muscles are stretched during stance one possible source for the afferent signal is stretch-sensitive receptors in these muscles. One of the main findings of the present study was that imposed stretches of the IP muscle during stance produced an earlier onset of flexor activity (Figs. 4.1, 4.2 and 4.3). Stretches of the ankle flexors TA and EDL were also observed to produce similar effects on the timing of flexor burst activity (Fig. 4.2). The general conclusion of this study is that feedback from stretch-sensitive afferents in leg flexor muscles during stance acts to facilitate the transition to flexor activity associated with the swing phase of the locomotor cycle.

4.4.1. Action of flexor afferents during extension.

In general, the effects of stretching the hip and ankle flexor muscles on the timing of the locomotor rhythm were modest. Typically, the reduction in the duration of the extensor phase in which a stretch was applied was between 10 and 20% (Fig. 4.2). There exists the possibility that the magnitude of the reduction in the extensor phase was usually limited by strong mutual inhibition between the flexor half-centres (for review of half-centre hypothesis for locomotion see Lundberg 1980) of the central rhythm generator (Orsal et al. 1990). In most instances the stretches were applied during the flexion phase of the contralateral leg (Fig. 4.3A). Thus, the ipsilateral flexor burst could not be generated immediately after the onset of the stretch because of inhibition from the contralateral flexor half-centre. Consistent with this conclusion is that the timing of the

onset of the ipsilateral flexor bursts was strongly correlated with the termination of the contralateral flexion phase (Fig. 4.3C). Furthermore, when stretches were applied during the contralateral extensor phase there was a much earlier onset of ipsilateral flexor activity and a corresponding greater reduction in extensor duration (Fig. 4.3 B and C). Theoretical considerations also support this conclusion. Assuming that the step cycles for each limb are 180^0 out of phase and that a flexor burst occupies one-third of the cycle it was calculated that the maximal reduction in extensor burst duration, when the stimulus onset occurs during contralateral flexion, should be 16.7% (provided that there is no change in flexor duration of the contralateral leg). This value is close to the average reduction in extensor burst duration observed during stretch of IP, TA and EDL in the immobilized limb preparations (Fig. 4.2B). The strong inhibitory influence of the contralateral flexor system has also been documented in another study which showed that flexion responses to unexpected perturbations during locomotion cannot be initiated if the contralateral limb is in swing phase (Gorassini et al. 1994). However, the inhibitory action between flexor burst generators can not be considered to be absolute. In the present study, while a synchronous activation of flexors from both hindlimbs never occurred, the early flexor activity in the test limb did occassionally overlap with the end of flexor activity in the contralateral limb (see Fig. 4.4B). This could represent a transient change from the alternating gait of walking to an in-phase gait such as galloping (Wetzel and Stuart 1976).

An unresolved issue is the mechanism by which the stretch-sensitive flexor afferents influence the locomotor rhythm generators. One clue is that stretch of the flexor muscles and electrical stimulation of flexor nerves produced a sustained inhibition of extensor activity with a latency of approximately 30 ms. This latency is much less than the latency to the onset of flexor bursts evoked during the contralateral extensor phase (95ms; Fig, 4.3C). These observations suggest that the flexor stretch-sensitive afferents act by inhibiting the extensor half-centre (pathway 1 in Fig. 4.11) and that the earlier onset of flexor activity is a secondary consequence of less inhibition from the extensor to the flexor half-centre. Of course, this does not exclude the possibility of a direct excitatory action on the flexor half-centre (pathway 2 in Fig. 4.11). In addition to

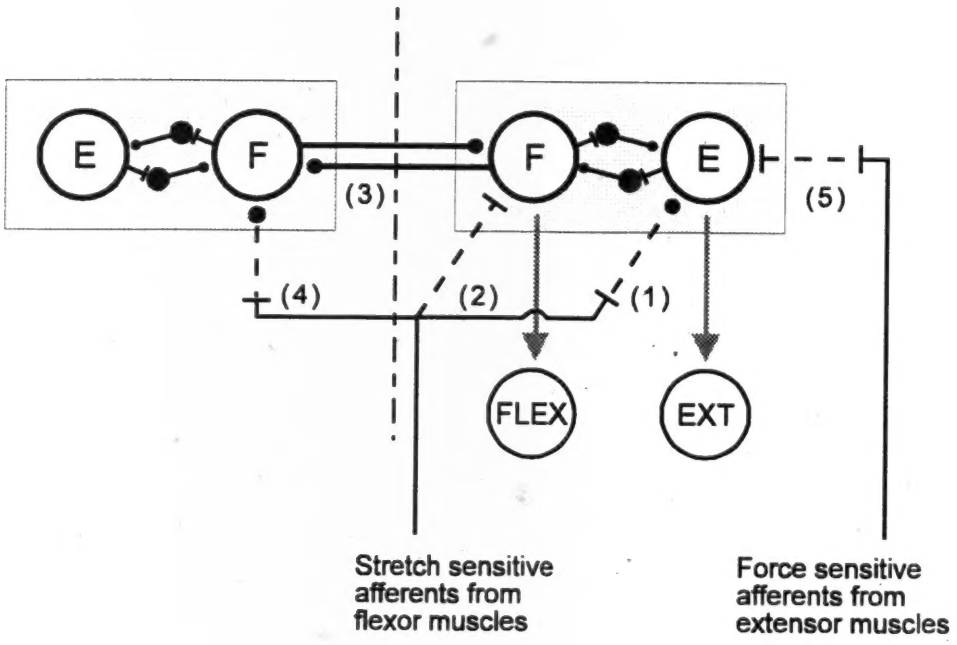

Figure 4.11. Schematic diagram illustrating the possible connections of stretch sensitive afferents from the flexor muscles onto spinal rhythm generating networks. The spinal rhythm generator for each hind leg is represented by mutually inhibiting extensor and flexor half-centres (shaded boxes), which project to flexor (FLEX) and extensor (EXT) motoneuronal pools. The black dots represent inhibitory connections, while the bars represent excitatory synapses. The dashed lines indicate possible polysynaptic pathways. We propose that the main effect of ipsilateral input from the stretch-sensitive flexor muscle spindle afferents (group Ia and/or II) is to inhibit the ipsilateral extensor half-centre thus allowing the flexors to become active (1). The possibility of an excitatory input to the flexor half-centre is also illustrated (2). Inhibition of the contralateral flexor activity is assumed to occur via the coupling of the ipsilateral and contralateral rhythm generating networks (3) in conjunction with a crossed inhibitory pathway from the stretch-sensitive afferents (4). The afferent input from force sensitive afferents from extensor muscles is assumed to excite the ipsilateral extensor half-centre (5).

influencing the timing of the rhythm in the ipsilateral leg, stretching the flexor muscles and electrically activating flexor muscle afferents usually reduced the duration of contralateral flexor phase (Figs. 4.3 and 4.4). The simplest explanation for this is an inhibitory connection to the contralateral flexor half-centre (pathway 3 in Fig. 4.11). Alternatively, the earlier onset of ipsilateral flexor activity may prematurely terminate the contralateral flexor bursts by the inhibitory pathway from the ipsilateral to contralateral flexor half-centres (pathway 4 in Fig. 4.11).

4.4.2. Identity of afferents promoting onset of flexor bursts.

A number of observations indicate that afferents arising from the spindles of the flexor muscles were responsible for reducing extensor duration and promoting the early onset of flexor activity. First, the reduction in extensor duration produced by EDL during electrical stimulation occurred at low strengths (1.2 xT). These stimulus levels would preferentially activate primary (group Ia) spindle afferents. Consistent with this was the observation that vibration of EDL also promoted an earlier onset of flexor activity, with the magnitude of the effect being similar to that produced by stretch (Figs. 4.2 and 4.4). Second, vibration of IP also promoted an earlier onset of flexor bursts, and repetitive bursts of vibration to this muscle could entrain the locomotor rhythm (Figs. 4.4 and 4.8). These observations suggest that the group Ia muscle spindle afferents from IP and EDL were generating the resetting effects.

For TA, however the data indicate that the secondary (group II) muscle spindle afferents must also be activated in order to promote the early onset of flexor activity when TA is stretched. First, resetting of the locomotor rhythm by group Ia afferents alone can be excluded because vibration of TA had no effect on the timing of the rhythm (Fig. 4.6C) and no effects on timing were observed when the TA nerve was stimulated to maximally activate group I afferents (approximately 2 xT; Jack 1978) (Fig. 4.5 D-F). Secondly group II muscle spindle afferents are progressively recruited over a range of stimulus strengths from 2 to 5 xT which corresponds to the observation of a progressively larger influence of TA nerve stimulation as the strength was increased over this range (Fig. 4.5 D-F). While these observations suggest that the activation of the

secondary group II muscle spindle afferents are responsible for TA's ability to reset the locomotor rhythm, they do not preclude the possibility that the primary group Ia spindle afferents may have a subthreshold effect. Therefore, it must be concluded that during stretch of the TA muscle group II muscle and possibly group I afferents are responsible for promoting the transition from extension to flexion.

The reason why the effects of TA and EDL stimulation are mediated by different sets of afferents is unclear in that both muscles have a similar function in flexing the ankle joint, with EDL having the additional role of extending the phalanges (toes). However, the primary (group Ia) muscle spindle afferent discharge that occurs at the end of stance phase is larger for EDL than for TA (Loeb and Duysens 1979). Also, there is evidence that the EDL nerve has a slightly greater divergence of monosynaptic connectivity onto other synergistic ankle flexors than the TA nerve (Eccles et al. 1957). It has been suggested that divergence of the monosynaptic reflex is mirrored by a parallel divergence onto interneurones mediating reciprocal inhibition of antagonistic motoneuronal pools (Baldisserra et al. 1981: Hultborn and Udo 1972). Based on these observations, the Ia afferents from EDL might also have a greater inhibitory effect on interneurons of the extensor half-centre.

Whether or not spindle afferents (group Ia and/or II) are solely responsible for the resetting effects of flexor muscle stretch is unknown. However, it is unlikely that input from the flexor muscle group Ib afferents, from force-sensitive Golgi tendon organs, is involved. In these experiments the stretch of the flexor muscles was typically applied during the period of extensor activity, ie. at a time when the flexor muscles were inactive. Because group Ib afferents respond primarily to forces produced in actively contracting muscles (Houk and Henneman 1967) it is unlikely that the flexor group Ib afferents would have been strongly activated by the muscle stretches.

Another possibility is that higher threshold group III afferents could facilitate the generation of flexor bursts since some are known to be stretch sensitive (Mense and Meyer 1985) and these afferents are usually included in the flexor reflex afferents (FRA) system (McCrea 1992). However, the observation that the magnitude of resetting was similar when flexor muscles were stretched and when flexor nerves were stimulated at

strengths below that necessary to activate group III afferents suggests that the effect of these afferents during stretch is probably minimal.

4.4.3. Action of flexor afferents during flexion.

The action of flexor muscle afferents on the timing of the locomotor rhythm has been examined in relatively few studies. Conway et al. (1987) reported that stimulation of the group I afferents from flexor afferents (exact nerves unspecified) did not produce phase shifts of the locomotor rhythm. A recent and more detailed investigation of flexor afferents also reported that stimulation of the group I afferents from flexor nerves during flexor activity did not alter the fictive locomotor rhythm (Perreault et al. 1995). This is consistent with the observation that stimulation of group I afferents from TA and that stretch of the IP muscle had no effect on flexor burst duration. However, in the present investigation stimulation of EDL group I afferents during flexor activity consistently prolonged the duration of the flexor bursts (Fig. 4.9A). The effect of stimulating afferents in the EDL nerve was not investigated in previous studies.

A major finding of Perreault et al. (1995) was that stimulation of the group II afferents from TA, sartorius, and semitendinosus during flexor activity consistently reset the locomotor rhythm to extension. In light of this earlier result, it was unexpected that stimulation of group II afferents from TA, in three of four experiments, resulted in no change in the duration of flexor activity, and that in one experiment stimulation of TA nerve at 5 xT actually prolonged the flexor burst in other flexor muscles. Furthermore, stimulation of EDL group II afferents during flexion produced a larger prolongation of flexor burst duration than that seen when the group I afferents were stimulated alone: typically the flexor burst was prolonged until the end of the stimulus train (Fig. 4.9B). Thus, in summary, the data in decerebrate walking animals indicate that flexor muscle afferents have either no effect or an excitatory effect on flexor burst generation.

At present, we can not account for the difference between these results and those of Perreault et al. (1995). However, it may be related to differences in the experimental preparations. The preparation used by Perreault et al. (1995) was immobilized and locomotion was evoked solely by electrical stimulation of the mesencephalic locomotor

region. Interestingly, in one preparation a transient inhibition of flexor activity in response to stimulating the EDL nerve at 5 xT was observed (Fig. 4.9B). This may reflect the inhibitory pathway described by Perreault et al. It is possible that in walking preparations this inhibitory pathway is weaker and can be overwhelmed by excitation mediated by other pathways.

4.4.4. Flexor muscle afferents facilitate the corrective response to the absence of ground support.

In Chapter 2, the postulate was put forth that the rapid onset of the flexor activity in response to a limb entering a hole in the supportive surface was primarily a result of the extensor muscles not being reinforced by loading (tendon organ group Ib feedback) and thus allowing an early flexor activation. This was demonstrated to be true in Chapter 3. However, a second factor that may contribute to the onset of the flexion response (proposed in Chapter 2) may be the afferent signal resulting from the rapid extension of the limb into the hole. Before the onset of the flexion response in chronic spinal cats the hip angle typically extended back to around 110 degrees before the flexion response occurred. This is very similar to the observation made by Grillner and Rossignol (1978) during passive extension of a limb.

Activity in IP typically occurs after ~165 ms when the hindlimb of a decerebrate cat encounters a hole in the supportive surface. In the present set of experiments, flexor activity occurred after 205 ms. This increase in latency is possibly a result of having cut the insertion of the IP muscle or the nerve to EDL, thus removing the normally occuring afferent feedback resulting from the rapid extension of the limb into the hole. The restoration of the "normal" afferent signal, by stretching the muscle or electrically activating the nerve, resulted in the latency of the flexion being closer to that previously established for decerebrate cats. These experiments demonstrate that stretch sensitive afferents from flexor muscle response contribute, along with loss of force feedback (Chapter 3), to onset of the corrective response to the absence of ground support.

4.4.5. Integration of proprioceptive signals.

The primary objective of this investigation was to establish whether stretch-sensitive afferents in the hind leg flexor muscles could be involved in triggering the transition from stance to swing. Although the data support this proposal, it is clear that other processes also regulate the timing of the swing phase onset. As mentioned in the Introduction, the most prominent of these is the load carried by the extensor muscles. Numerous studies have shown that activation of group I afferents in extensor muscles during stance inhibits the generation of flexor burst activity and promotes extensor activity (Conway et al. 1987; Guertin et al. 1995). In particular, there is evidence that a necessary condition for swing to be initiated is an unloading of the leg extensor muscles (Conway et al 1987; Pearson & Collins 1993). Muscle loading is signalled mainly by feedback from the force-sensitive Golgi tendon organs. Therefore, for the stretch-sensitive afferents in the flexor muscles to have a facilitatory action in promoting swing, the load carried by the leg must be below some critical level.

Consistent with this idea was the finding that stretching the IP muscle during quadrupedal walking produced a smaller reduction in the duration of extensor activity than that produced when the leg was immobilized (Fig. 4.2C). During quadrupedal locomotion the extensor muscles of the test limb were being phasically loaded. As stated above, force feedback during locomotion promotes extensor activity. Thus, for any reduction in extensor duration to occur, the inhibition produced by the stretch sensitive afferents from the flexor muscles must be greater than the excitation produced by loading of the extensor muscles. In the fixed leg preparation the latter was minimal because the tendinous insertion of the quadriceps and triceps surae muscle groups had been cut. It follows therefore that in this preparation activation of the flexor afferents would be expected to have a more potent effect in reducing the extensor duration, as indeed we observed.

Figure 4.11 summarizes the factors discussed that can influence the timing of the transition from extension to flexion during locomotion: inhibition of the extensor half-centre (1) and excitation of the flexor half-centre (2) by stretch-sensitive afferents in flexor muscles, mutual inhibition of the flexor half-centres (3), crossed inhibition of the

contralateral flexor half-centre (4), and indirect inhibition of the flexor half-centre by the Golgi tendon organs of extensor muscles reinforcing the extensor half-centre (5). Clearly the scheme illustrated in figure 4.11 provides only a partial summary for the mechanisms that normally function to regulate the initiation of swing. It also must be considered that cutaneous afferents may contribute to the control of phase transitions in the step cycle. There are numerous examples of the cutaneous afferent input modifying the locomotor pattern (Duysens and Pearson 1976; Duysens 1977; Wand et al. 1980; Andersson et al. 1978; Drew and Rossignol 1987).

Also not illustrated in figure 4.11 are the possible connections to and contributions of supraspinal structures. Given the long latency of the inhibition of the extensor EMG (20-40ms) and the even longer latency to the onset of the flexor activity (95ms; see Fig. 4.3C) the activation of supraspinal structures could be involved in generating the effects documented in the present study. For example, in decerebrate animals signals from the reticular formation and red nucleus can strongly influence the flexor burst generating system (Armstrong 1986) and in intact animals descending signals from the motor cortex have also been shown to facilitate flexion (Drew 1991, 1993; Armstrong 1986).

Finally, the overall role of peripheral feedback in the regulation of the walking pattern may be very dependent on the type of preparation used (ie. fictive locomotion compared to decerebrate walking compared to intact walking). Already there are examples of different responses produced in different preparations. One example from this study is that stimulation of the ankle flexors during flexor activity can prolong the duration of the flexor bursts, whereas stimulation in a fictive preparation generates a resetting to extensor activity (Perreault et al. 1995). Another is the absence of effect of extensor group Ia afferents on the locomotor rhythm in spinal animals (Conway et al. 1987; Pearson et al. 1992) and a marked effect on the fictive rhythm in decerebrate preparations (Geurtin et al. 1995). Nonetheless, studies such as these bring us closer to understanding the complex interactions of afferent feedback and the neural circuitry producing locomotor activity.

4.5. References.

Andersson, O., Forssberg, H., Grillner, S., and Lindquist, M. Phasic gain control of the transmission in cutaneous reflex pathways to motoneurones during `fictive' locomotion. *Brain Res.* 149:503-507, 1978.

Andersson, O. and Grillner, S. Peripheral control of the cat's step cycle: II. Entrainment of the central pattern generators for locomotion be sinusoidal hip movements during `fictive locomotion'. *Acta Physiol. Scand.* 118:229-239, 1983.

Armstrong, D. M. Supraspinal contributions to the initiation and control of locomotion in the cat. In: *Progress in Neurobiology: Vol.26*, Great Britain: Pergamon Journals Ltd., 1986, p. 273-361.

Baldissera, F., Hultborn, H., and Illert, I. Integration in spinal neuronal systems. In: *Handbook of physiology: the nervous system II*, 1981, p. 509-595.

Brown, M. C., Engberg, I., Mathews, P. B. C. The relative sensitivity to vibration of muscle receptors of the cat. *J. Physiol.* 192:773-800, 1967.

Conway, B. A., Hultborn, H., and Kiehn, O. Proprioceptive input resets central locomotor rhythm in the spinal cat. *Exp. Brain Res.* 68:643-656, 1987.

Drew, T. Visuomotor coordination in locomotion. *Curr. Opin. Neurobiol.* 1:652-657, 1991.

Drew, T. Motor cortical activity during voluntary gait modification in the cat. I. Cells related to the forelimb. *J. Neurophysiol.* 70:179-199, 1993.

Drew, T. and Rossignol, S. A kinematic and electromyographic study of cutaneous reflexes evoked from the forelimb of unrestrained walking cat. *J. Neurophysiol.* 57:1160-1183, 1987.

Duysens, J. Reflex control of locomotion as revealed by stimulation of cutaneous afferents in spontaneously walking premamillary cats. *J. Neurophysiol.* 40:737-750, 1977.

Duysens, J. and Pearson, K. G. The role of cutaneous afferents from the distal hindlimb in the regulation of the step cycle of thalamic cats. *Exp. Brain Res.* 24:245-255, 1976.

Duysens, J. and Pearson, K. G. Inhibition of flexor burst generation by loading ankle extensor muscles in walking cats. *Brain Res.* 187:321-332, 1980.

Eccles, J. C., Eccles, R. M, and Lundberg, A. The convergence of monosynaptic excitatory afferents on to many different species of alpha motoneurones. *J. Physiol.* 137:22-50, 1957.

Forssberg, H. and Grillner, S. The locomotion of the acute spinal cat injected with clonidine i.v. *Brain Res.* 50:184-186, 1973.

Gorassini, M. A., Prochazka, A. K., Hiebert, G. W., and Gauthier, M. J. A. Corrective responses to loss of ground support during walking I. Intact cats. *J. Neurophysiol.* 71:603-610, 1994.

Goslow, G. E., Stauffer, E. K., Nemeth, W. C., and Stuart, D. G. The cat step cycle: responses of muscle spindles and tendon organs to passive stretch within the locomotor range. *Brain Res.* 60:35-54, 1973.

Grillner, S. Control of locomotion in bipeds, tetrapods, and fish. In: *Handbook of physiology: the nervous system II*, edited by V. B. Brooks. Bethesda, MD: American Physiology Society, Waverly Press, 1981, p. 1179-1236.

Grillner, S. and Rossignol, S. On the initiation of the swing phase of locomotion in chronic spinal cats. *Brain Res.* 146:269-277, 1978.

Grillner, S. and Zangger, P. The effect of dorsal root transection on the efferent motor pattern in the cat's hindlimb during locomotion. *Acta. Physiol. Scand.* 120:393-405, 1984.

Guertin, P., Angel, M., Perreault, M. -C., and McCrea, D. A. Ankle extensor group I afferents excite extensors throughout the hindlimb during MLR-evoked fictive locomotion in the cat. *J. Physiol.* 487(1):197-210, 1995.

Horsmann, U., Heinzel, H. -G., and Wendler, G. The phasic influence of self-generated air current modulations of the locust flight motor. *J. Comp. Physiol.* 150:427-423, 1983.

Houk, J. C. and Henneman, E. Responses of Golgi tendon organs to active contractions of the soleus muscle of the cat. *J. Neurophysiol.* 30:466-481, 1967.

Hultborn, H. and Udo, M. Convergence of large muscle spindle (Ia) afferents at interneuronal level in the reciprocal Ia inhibitory pathway to motoneurones. *Acta Physiol. Scand.* 84:493-499, 1972.

Jack, J. J. B. Some methods for selective activation of muscle afferent fibres. In: *Studies in Neurophysiology*, edited by R. Porter. Cambridge: Cambridge University Press, 1978, p. 155-176.

Kriellaars, D. J., Brownstone, R. M., Noga, B. R., and Jordan, L. M. Mechanical entrainment of fictive locomotion in the decerebrate cat. *J. Neurophysiol.* 71:2074-2086, 1994.

Loeb, G. E. and Duysens, J. Activity patterns on individual hindlimb primary and secondary muscle spindle afferent during normal movements in unrestrained cats. *J. Neurophysiol.* 42(2):420-440, 1979.

Lundberg, A. Half-Centres Revisited. In: *Regulatory Functions of the CNS. Motion and Organizational Principles*, edited by J. Szentagothai, M. Palkovits and J. Jamori. Pergamon Press, 1980, p. 155-167.

McCrea, D. A. Can sense be made of spinal interneuron circuits? *Behav. Brain Sci.* 12:633-643, 1992.

Mense, S. and Meyer, H. Different types of slowly conducting afferent units in cat skeletal muscle and tendon. *J. Physiol.* 363:403-417, 1985.

Orsal, D., Cabelguen, J. -M., and Perret, C. Interlimb coordination during fictive locomotion in the thalamic cat. *Exp. Brain Res.* 82:536-546, 1990.

Pearson, K. G., Ramirez, J. M., and Jiang, W. Entrainment of the locomotor rhythm by group Ib afferents from ankle extensor muscles in spinal cats. *Exp. Brain Res.* 90:557-566, 1992.

Pearson, K. G. and Collins, D. F. Reversal of the influence of group Ib afferents from plantaris on activity in medial gastrocnemius muscle during locomotor activity. *J. Neurophysiol.* 70:1009-1017, 1993.

Perreault, M. -C., Angel, M., Guertin, P., and McCrea, D. A. Stimulation of hindlimb flexor group II afferents during fictive locomotion. *J. Physiol.* 487(1):211-220, 1995.

Prochazka, A., Trend, P. St. J., Hulliger, M., and Vincent, S. Ensemble proprioceptive activity in the cat step cycle: towards a representative look-up chart. *Prog. Brain Res.* 80:61-74, 1989.

Shik, M. L., Severin, F. V., and Orlovsky, G. N. Control of walking and running by means of electrical stimulation of the mid-brain. *Biophysics* 11:756-765, 1966.

Wand, P., Prochazka, A., and Sontag, K. -H. Neuromuscular responses to gait perturbations in freely moving cats. *Exp. Brain Res.* 38:109-114, 1980.

Wetzel, M. C., Stuart, D. G. Ensemble characteristics of cat locomotion and its neural control. *Prog. Neurobiol.* 7:1-98, 1976.

CHAPTER 5.

The contribution of sensory feedback to the generation of extensor activity during walking in the decerebrate cat.

5.1 Introduction.

The function of peripheral afferents, in particular those arising from muscle receptors, has been an important issue in the physiology of locomotion since the early work of Sherrington (1910). In recent years, a primary focus has been on the role of afferent feedback in controlling the timing of the transition from stance to swing. There is evidence that activation of large muscle afferents, arising from muscle spindles and tendon organs in extensor muscles, is capable of regulating the duration of extensor activity associated with stance phase (Chapter 3 of this thesis; Conway et al. 1987; Whelan et al. 1995a; Guertin et al. 1995). Also, proprioceptive input from the muscle spindle afferents generated by the stretch of flexor muscles as the limb is extended during stance has been shown to bring about an early transition from stance to swing (see Chapter 4). These observations indicate that afferent feedback from muscle receptors signaling loading of the leg and extension of the leg are both involved in controlling the timing of the phase transitions.

Another possible role of afferent feedback is in the generation of the EMG activity within a single phase of the locomotor cycle. The idea that monosynaptic excitatory feedback from muscle spindle afferents may contribute to muscle activity during stance arose when Severin, Orlovskii and Shik (1967) observed increased spindle activity during stance in treadmill walking decerebrate cats. Severin (1970) later provided evidence that afferent feedback produced by gamma activation of muscle spindles may contribute nearly 50% of the overall triceps activity. A recent study by Yang et al. (1991) also concluded that velocity sensitive afferents reflexively contribute 30-60% of the overall activity in the soleus muscle during walking in man. Other studies have shown that muscle spindles are involved in the generation of EMG activity during

voluntary contractions in man. Blockade of fusimotor drive acts to decrease the number of motoneurons recruited (Hagbarth et al. 1986) and reduce their firing rates by one-third (Macefield et al. 1993) during maximal voluntary contractions.

Another group of afferents that may contribute to the generation of stance activity is the group Ib afferents from extensors. Originally, the group Ib (force sensitive) afferents from the tendon organs were demonstrated to produce a disynaptic inhibition of homonymous and synergistic motoneurons (Laporte and Lloyd 1952; Eccles et al. 1957) and therefore not considered to reinforce muscle activity. However, over the past 15 years, evidence has accumulated that demonstrates that the disynaptic group Ib inhibition is weakened during locomotion and replaced by excitation. Conway et al. (1987) showed that activation of extensor group I afferents during fictive locomotion enhances the amplitude of extensor activity, and if stimulated during flexor activity, resets the locomotor rhythm to extensor activity. This effect was attributed to the group Ib afferents in that a similar response could not be generated by the activation of just the Ia afferents by vibration of the muscle tendon. The idea that the Ib inhibitory input observed in a passive, non-locomoting state switches to excitation during an active, locomoting state has now been conclusively demonstrated (Pearson and Collins 1993; Gossard et al. 1994). In addition, feedback from group Ia muscle spindles has also been recently shown to influence extensor activity through locomotor dependent disynaptic and oligosynaptic pathways (Guertin et al. 1995; McCrea et al. 1995). This has given rise to the possibility that during locomotion the group Ia and Ib afferents from extensor muscles converge onto a set of common interneurons which produce depolarization of synergistic motoneurons (see Fig 8 in McCrea et al. 1995).

While it is clear that muscle afferent feedback can influence the activity of motoneurons, either via the group Ia monosynaptic reflex or locomotion dependent group I polysynaptic pathways (Gossard et al. 1994; McCrea et al. 1995), there are still a number of unresolved issues. One is to establish the degree to which afferent feedback contributes to the generation of ankle and knee extensor activity during locomotion. The earlier study of Severin (1970) only made estimates in regards to the contribution of group Ia afferents in the generation triceps surae activity. Another issue is to determine

the extent to which the magnitude of EMG activity in separate muscle groups (ie. triceps surae - ankle extensors, as compared to quadriceps - knee extensors) is regulated independently. Guertin et al. (1995) have shown that afferent feedback from knee as compared to feedback from ankle extensors does not generate a similar pattern of extensor excitation. Thus it is of interest to examine whether or not the activity of certain muscle groups is more susceptible to local feedback or to general feedback from the entire limb. In this study we address these issues by selectively loading or unloading the hindlimb extensors of a decerebrate walking cat.

5.2 Methods.

Adult cats were anesthetized with halothane and the trachea cannulated for continued administration of the anesthetic. One of the carotid arteries was ligated and a cannula to monitor the blood pressure was placed in the other. A cannula was inserted in one jugular vein for the administration of fluids and drugs. Bipolar electromyogram (EMG) electrodes were sutured into various flexor and extensor muscles of the hind legs: lateral gastrocnemius (LG), soleus (SOL), vastus lateralis (VL), vastus medialis (VM), iliopsoas (IP), semitendinosus (ST) and/or tibialis anterior (TA). In some animals E-shaped force-buckles were placed on the patellar tendon and/or Achilles tendon (see Prochazka 1984).

Prior to decerebration, the animal was prepared for one of the four experimental paradigms described below. Decerebration was performed by transecting the brainstem at a 50° angle from the anterior edge of the superior colliculus, leaving the mammillary bodies intact. After the decerebration the anaesthetic was discontinued and within one hour spontaneous locomotion activity usually occurred. If no spontaneous walking occurred, stimulation of the mesencephalic locomotion region (MLR) was used to induce locomotion (15 Hz, 0.6 msec duration, 30-100 mA; co-ordinates P2,L4,H6; Shik et al. 1966).

All procedures were carried out with approval from the University of Alberta Health Sciences Animal Welfare Committee.

5.2.1. Extensor activity during the absence of ground support

In Chapter 3, a preparation was used in which a decerebrate walking cat encountered a hole in one side of the treadmill belt upon which it was stepping. In this situation it was observed that approximately 85% of the time the animals exhibited a termination of extensor activity and a rapid onset of flexor activity which removed the limb from the hole. In the remaining 15% of the encounters with the hole, the limb was held extended in the hole for a prolonged period. The EMG activity of the hindlimb muscles during the trials in which no flexion response occurred were examined in the present investigation (see Fig. 5.1). This data was not included in Chapter 3.

5.2.2. Feedback from ankle extensor muscles

In 5 animals the amount of force generated by the ankle extensors was controlled when the animal encountered a hole in the treadmill belt (see Fig. 5.3). A wire was attached to a clamp secured to the dorsum of the foot (the superficial and deep peroneal and the distal tibial nerves were cut to denervate the foot). The wire passed through a guide that was attached to the tibia. The other end was attached to a DC servo motor. The distance between the tibial attachment and the motor was maintained by a sheath inside of which the wire could freely move. Thus changing the position of the motor resulted in a change of ankle position. Tension in the wire was monitored and compared to a desired level of background force being controlled by a computer. The motor acted to adjust the tension to match a preset value. During locomotion the motor was typically set to maintain low tension in the wire (ie. 25g). Thus the motor would closely follow the normal flexion and extension movements of the ankle joint during walking. When the limb entered a hole in the treadmill surface the level of required force in the guide wire was rapidly changed to a new level for a set period of time, and then returned to the normal level. Entrance of the limb into the hole was monitored by a series of light emitting diodes located just beneath the treadmill surface (see Chapter 2).

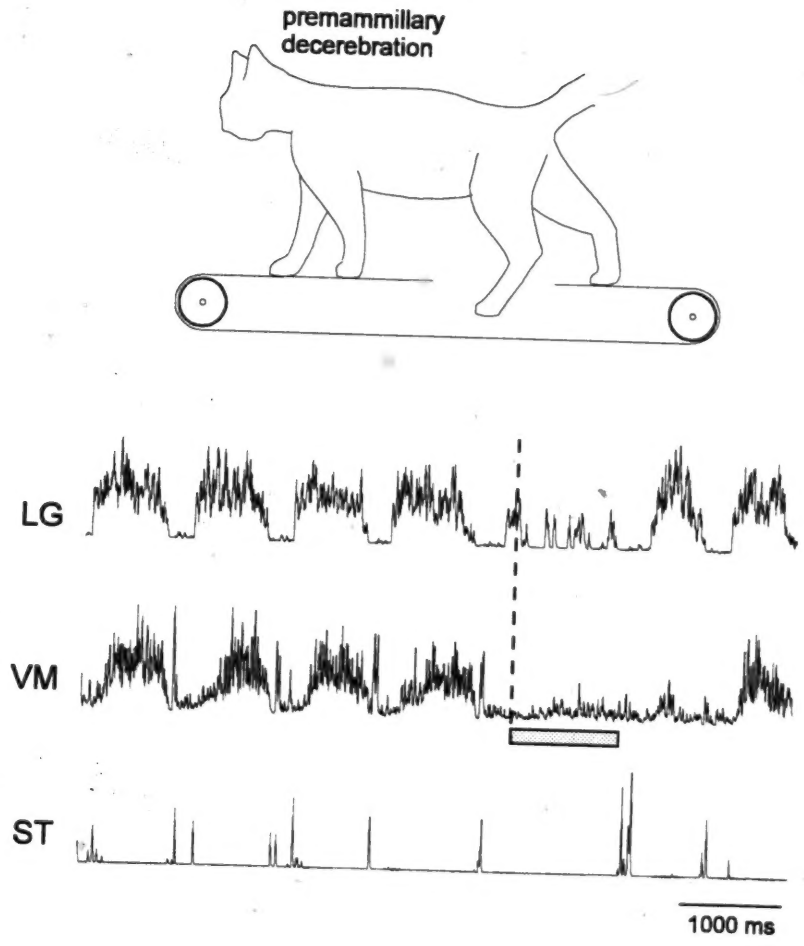

Figure 5.1. Illustration of a cat encountering a hole in the surface of a moving treadmill belt and the EMG activity when the animals fails to produce a corrective flexion reaction. The dashed vertical indicates the time the limb entered the hole and the shaded bar indicates the period that the limb was extended in the hole. Note the large reduction in the magnitude of EMG activity in the ankle (LG) and knee (VM) extensors when the limb enters the hole.

5.2.3. Loading or unloading of the hindquarters during walking

In four animals the L7 to S2 spinous processes were exposed and a length of strong surgical thread passed through the base of each spine. The thread was subsequently attached to a DC servo motor mounted above the animal. Custom written computer programs were used to recognize the onset of EMG bursts, which were then used to trigger a controlled lift or drop (~3cm) of the hindquarters.

5.2.4. Extensor activity following partial deafferentation

The spinal cord of four animals was exposed by laminectomy of the 4th - 7th lumbar (L4-L7) vertebrae. The dura mater was then cut and the nerve fascicles of the individual left dorsal roots (L4 - S2) were identified and loosely grouped together with surgical thread. After recording the EMG activity during decerebrate locomotion in the intact state, the dorsal roots on one side were sequentially transected (beginning at L4 and ending with S2), and a segment of walking recorded after each root had been cut. In 3 of 5 animals elastic straps were used to maintain lateral stability of the animal. Occasionally, elastics were used to assist in vertical weight support, although for the most part the animals were capable of supporting their own weight.

5.2.5. Data analysis

Surface markers over the iliac crest, greater trochanter, lateral epicondyle of the femur, lateral malleolus and metatarso-phalangeal joint and the relative joint angles of the leg monitored with a video recorder (66 frames/s). Individual step cycles were then saved on computer disc using Video-Blaster software (Creative Labs, Inc.). Custom written software was then used to display the kinematics and calculate the joint angles of the limb. Microsoft Excel software was used to generate the angle plots.

Custom written software capable of reading and displaying the Axotape data files was used to display and store averaged EMG data. Microsoft Excel software was used to calculate the average extensor EMG activity during a single step cycle for a 500 ms period beginning 100 ms after the onset of activity in SOL for an individual step. The initial 100 ms was not included because this period corresponds to the E1 phase of the

locomotion step cycle where the limb is not in contact with the ground. The extensor EMG activity during the E1 phase is not dependent on the afferent signals generated by foot contact (Gorassini et al. 1994). The average activity and standard deviation for a number of step cycles was then calculated. The activity during undisturbed walking was taken to be 100% and the level of EMG activity during a perturbation to the stance phase calculated as a percentage of the control. A Student's T-test (unpaired) was used calculate significant changes from the control.

5.3 Results.

5.3.1. Extensor EMG activity is reduced during the absence of ground support

In the previous Chapters, it was reported that when a hindlimb of a walking cat encounters a hole in the supporting surface that a very distinct corrective reaction occurs. Typically, in the decerebrate animal during an encounter with the hole extensor activity ceases and flexor activity is initiated approximately 170 ms after the limb enters the hole (see Chapter 3). The flexor activity acts to remove the foot from the hole. However, in the decerebrate cat preparation 15% of the trials do not exhibit the rapid onset of flexor activity. Instead the limb is held in an extended in the hole. Using these trials it was possible to assess the contribution of afferent feedback in the generation of extensor activity.

Figure 5.1 illustrates the EMG activity when a decerebrate cat failed to produce a flexion reaction in response to a hole. Following the initial activity in LG, which is not dependent on the afferent feedback generated by limb contact with the ground (Gorassini et al. 1994), there was a large reduction of activity for the time the limb was held extended in the hole. A similar lack of activity occurred for the knee extensor VM. The pattern of EMG activity was very similar to that which occured when the onset of the flexion response out the hole was suppressed by providing group I (muscle spindle and tendon organ) afferent feedback by means of electrically stimulating the proximal stump of a severed extensor nerve (see Fig 3.2B in Chapter 3).

Figure 5.2 quantitatively illustrates the reduction in extensor EMG activity during the absence of a corrective flexion reaction. The average EMG activity during the failure to produce a corrective flexion reaction has been compared to the preceding two extensor bursts. The data is from three separate experiments. When the limb was held extended in the hole, extensor EMG activity was reduced to 29%, 16%, 17%, and 24% that of normal, for VL, VM, LG, and MG, respectively. Student's T-test (unpaired) indicated that all but one of these values represent a significant ($p<0.05$) reduction in extensor activity. The exception, VM, shows a similar trend, that would most likely be significant had the number of trials been greater. These results indicate that the normally occurring afferent feedback generated during stance phase is potentially responsible for almost 80% of the extensor EMG activity.

Loading extensor muscles restores EMG activity

While the above experiment demonstrates that afferent feedback generates a considerable portion of muscle activity, it does not reveal which afferents (ie. muscle spindle, tendon organs, or cutaneous receptors from the foot pad) are involved. In this subset of experiments, loading of the triceps surae during an encounter with the hole was used to selectively activate muscle afferents.

Only two of the five animals used in this set of experiments produced a useable walking pattern. Nonetheless, in the two animals that did step, the extensor EMG activity during the absence of the corrective flexion response was very similar to that reported in the previous section. Figure 5.3B illustrates the low level of activity in SOL and LG as the limb was held in the hole (note that the wire was not attached to the foot in this example).

There was no notable change in the EMG activity of the limb during normal walking when the wire was attached and the position motor was maintaining a low level of background force (25g) in the wire. This was taken to indicate that the wire was following the natural movement of the limb. When the limb encountered the hole the tension in the wire was increased (wire tension = 500g) so that force recorded in the Achilles tendon was close to that occurring during normal stance phase. Under these conditions EMG activity observed in SOL and LG was similar in magnitude to that

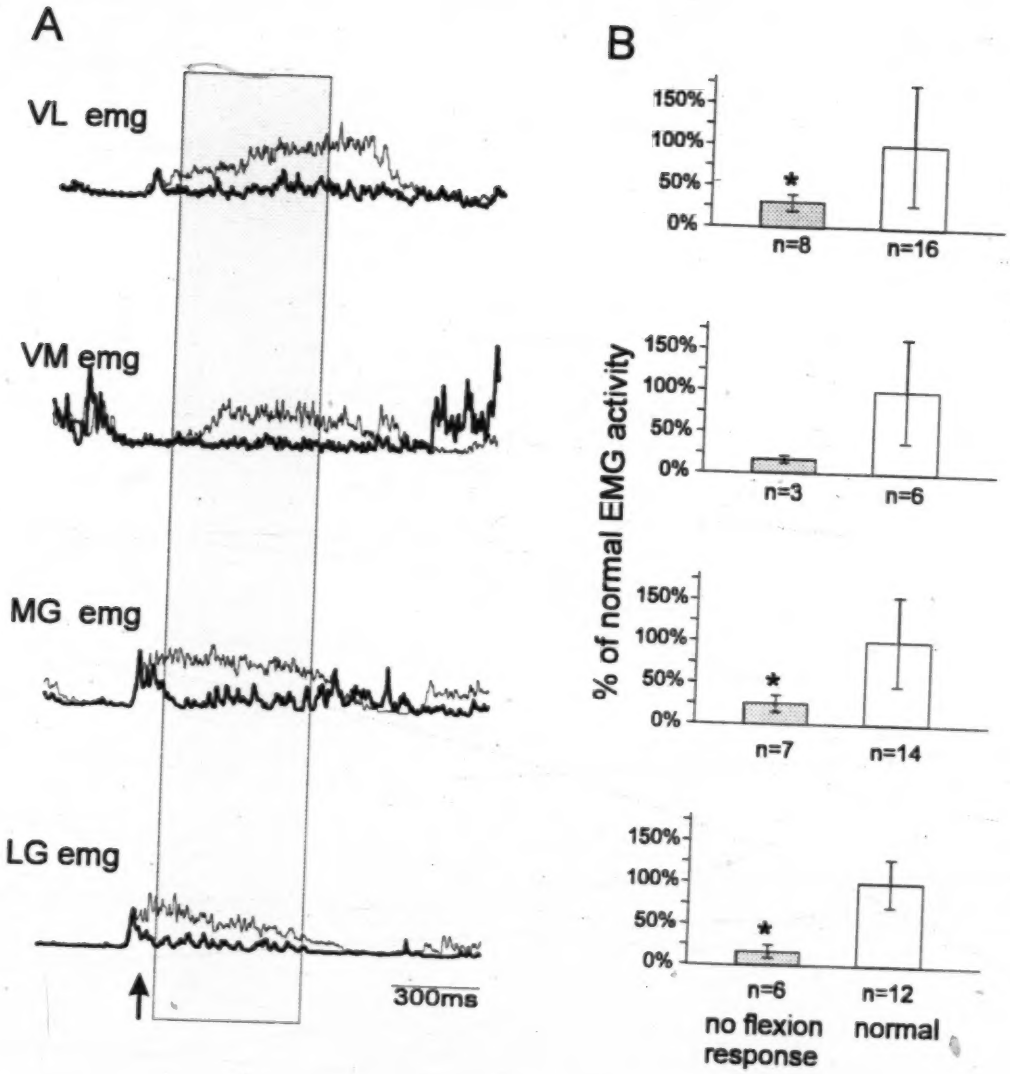

Figure 5.2. Rectified and averaged EMG activity during the failure to produce a corrective flexion reaction as compared to normal stance phase activity. The thin line is the EMG activity during undisturbed stance phase. The thick line is the activity when the limb is held extended in the hole (arrow indicates time the limb entered the hole). The average EMG amplitude has been calculated for a 500ms period (indicated by the shaded region) and is plotted in the histograms. Error bars indicate standard deviation. The extensor activity produced during unperturbed locomotion been taken as 100%. Without the presence of ground support (shaded bars) the EMG activity is significantly reduced to approximately 20% that of normal (asterisk = $p<0.05$).

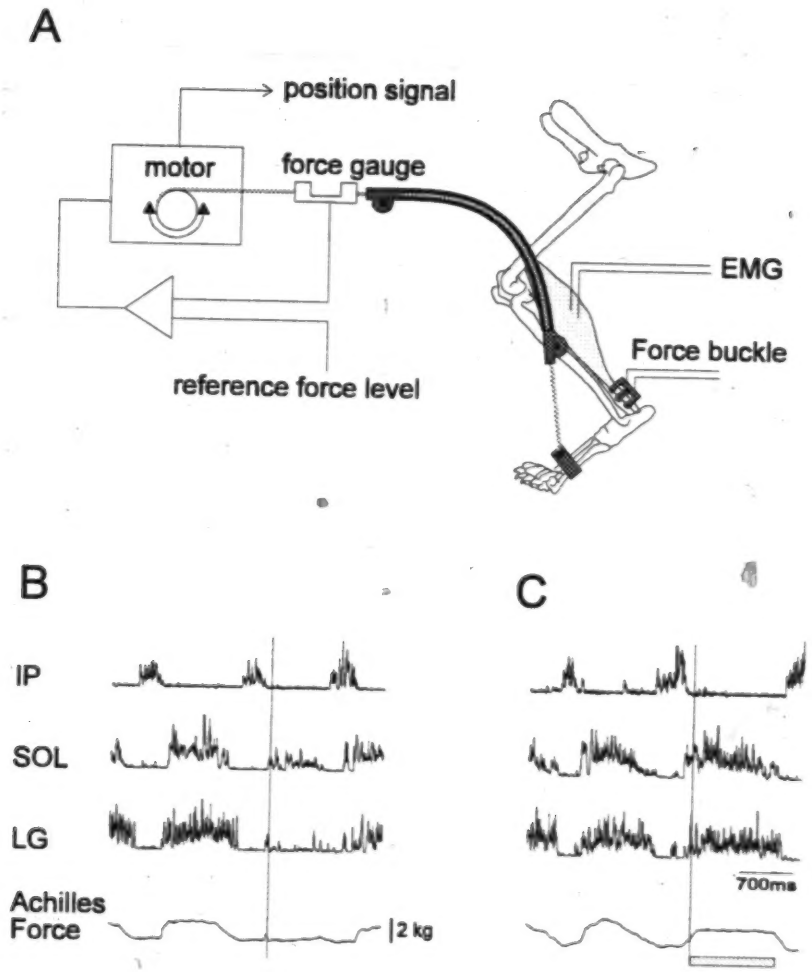

Figure 5.3. Illustration of the preparation used to control the amount of force being generated by the triceps surae muscle group as the limb encountered the absence of ground support (A). Rectified EMG traces when the limb failed to produce a flexion response (B), and when the tension in the wire increased from 25g to 500g when the limb entered the hole (C). The shaded bar indicates the period that the tension in the wire was increased. The vertical lines indicate the time the limb entered the hole (B) or the onset of the increased tension in the wire. See Methods and Results sections for details.

observed during weight-bearing steps, and distinctly greater in amplitude than that observed during the control trials where the wire was not attached (compare Fig. 5.3B and C). In addition to increasing the magnitude of bursts in the ankle extensors, loading the muscle also increased the duration of the bursts.

These results indicate that by selectively increasing proprioceptive feedback from muscle afferents (by loading the ankle extensor muscles) during an encounter with the hole that most of the normal level of EMG activity in the ankle extensor muscles can be generated. Whether loading of the ankle extensors as the limb entered the hole also increases the activity in knee extensors remains to be determined.

5.3.2. EMG changes during loading or unloading of the hindquarters

In numerous studies the excitatory effect of triceps surae muscle afferents onto extensors throughout the hindlimb has been demonstrated during fictive locomotion in the cat (Conway et al. 1987; Gossard et al. 1994; Guertin et al. 1995). In decerebrate walking cats the excitatory action of ankle extensor group I afferents on other extensor muscles has also been observed (Whelan et al. 1995a). However, the excitation induced by ankle extensor group I afferents is strongest onto other ankle extensors as compared to the response evoked in knee extensor muscles (Whelan 1996). Fewer studies have examined the action of quadriceps group I afferents on other extensors of the hindlimb, and those studies have produced mixed results with quadriceps afferents being weakly excitatory or inhibitory to ankle extensor muscles (Whelan et al. 1995a; Guertin et al. 1995). These earlier studies suggest that while afferent feedback is effective for generating activity in synergistic muscle groups (ie. LG onto MG, VL onto VM) it is not as effective in exciting functionally heteronymous muscles (ie. LG onto VL). In order to further investigate whether afferent feedback is segregated onto functionally synergistic muscles, the animals hindquarters was rapidly lifted or lowered to unload or load the quadriceps muscles during stance phase.

Figure 5.4 illustrates the changes in EMG activity and joint angles when the animal's hindquarters were held at different heights during walking. "Full weight support" represents the situation where the animal was walking unassisted; being forced

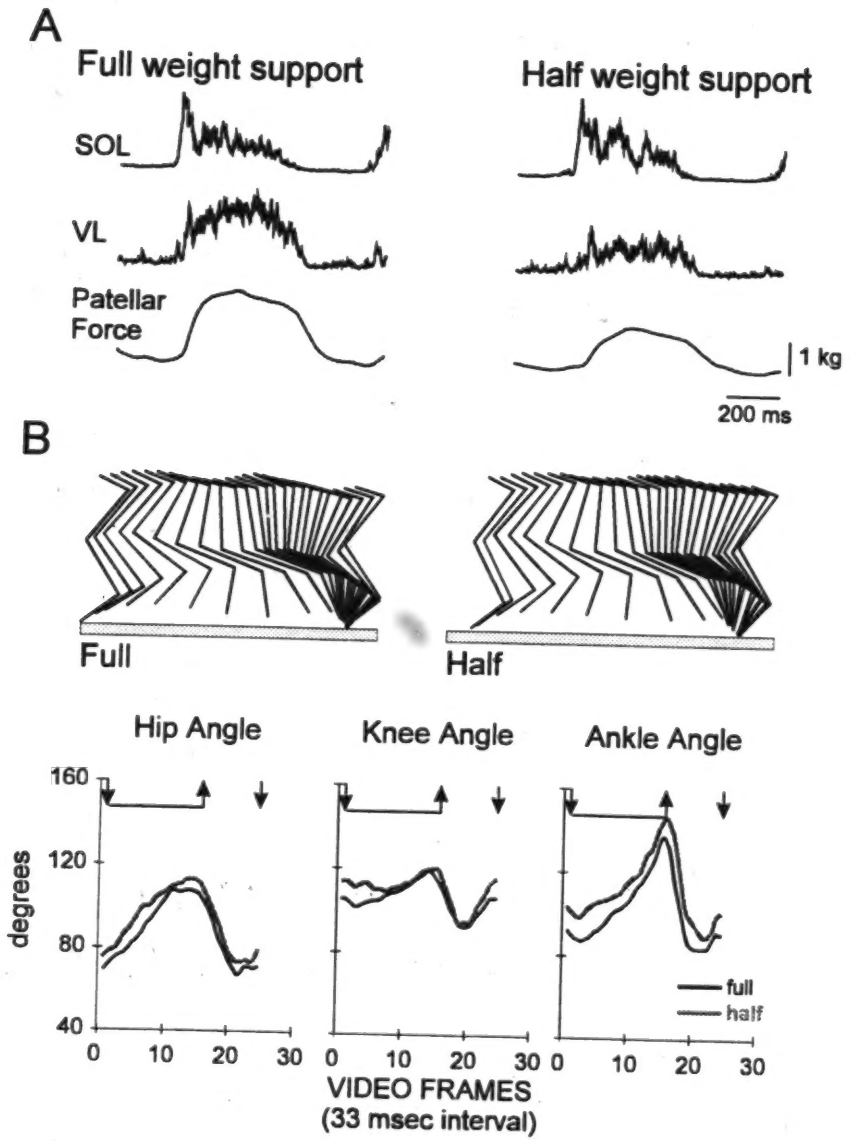

Figure 5.4. Average (n=5) EMG activity and kinematic pattern when the animal was held at different heights during walking. Data for Full weight support is represented by the thick black line and Half weight support is indicated by the grey colored line. A) VL activity and patellar force are reduced to half of that observed during Full weight support, whereas the activity in SOL is not noticeably changed. B) The step cycles used to generate the joint angle plots are taken from the same steps used for the average EMG. The arrows indicate the onset of swing and stance, with the solid line representing the duration of stance phase. Note that there was relatively little change in the joint angles.

to carry its own weight. During "Half weight support" the animals hindquarters were lifted 2cm, resulting in a decrease in patellar force to half that recorded for unassisted locomotion. There was a distinct decrease in the amplitude of VL EMG when the animal was lifted, but SOL activity was not noticeably affected (Fig. 5.4A). Figure 5.4B illustrates the kinematics from a single step cycle and the average (n=5) joint angles during Full and Half weight support locomotion. Note that the slight elevation of the animal above the treadmill did not produce a marked change in the joint angles during walking.

The observation that the quadriceps activity decreased as the force required for weight support decreased suggests that afferent feedback is acting to continuously regulate the level muscle activity to be sufficient to cope with the external demands. If this is true, then it was postulated that transient changes in the load carried by the animal should be mirrored by changes in EMG activity. Figure 5.5A illustrates the change in EMG activity following a 3cm lift, which unloads the hindquarters, triggered 50 ms after the onset of activity in SOL. Similar to the observations described above, a distinct feature was that EMG activity in the knee extensors was greatly reduced whereas there was a negligible effect in the ankle extensors. In one animal, where all of the quadriceps muscles were implanted with EMG electrodes, there was a general reduction of the quadriceps activity (data not shown). It was qualitatively noted that this reduction was greatest in VL and VM, whereas the deeper, more postural vastus intermedius muscle was the least affected by the lifting. In all 4 animals examined, there was no significant change in the amplitude of activity in SOL or LG during lifting of the animal.

A rapid 3cm drop of the hindquarters, mid way through stance phase, produced a distinct increase in VL activity 20-30 ms after the onset of the drop, which then lasted for the remainder of the stance phase. A transient increase in SOL activity was also observed 20-30 ms after the onset of the drop (arrow in Fig. 5.5B). However, following this short burst SOL EMG returned to the amplitude observed during undisturbed stepping.

Combining the ramp lift / lowering of the hindquarters produced a combination of the two above responses (Fig. 5.5C). The initial lifting of the hindquarters produced a reduction in VL activity that was identical to that illustrated in figure 5.5A. The rapid

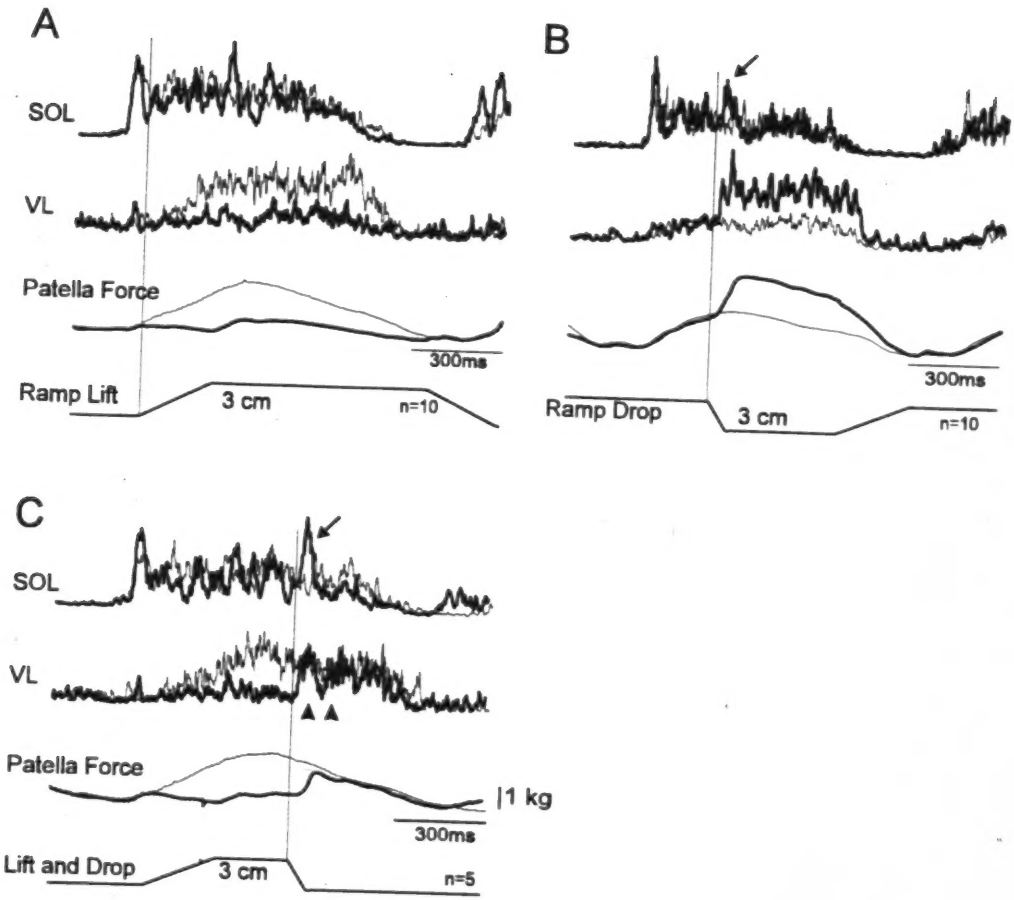

Figure 5.5. Rectified and averaged EMG activity of the ankle extensor SOL and the knee extensor VL during a ramp lift (A) or drop (B) of the hindquarters of a decerebrate walking cat. EMG activity in VL and the patella force are very dependent on the type of perturbation applied, being virtually absent during the lift and distinctly increased following dropping the hindquarters. The thin line represents the level of normal activity and the thick line perturbed EMG activity. Note that SOL activity is relatively unchanged, except for a transient increase in activity just after the onset of the drop (arrow in B). Combining the perturbations, by first lifting then midway into stance phase dropping the hindquarters (C), produced a combination of the two EMG responses. First EMG activity in VL is greatly reduced and upon loading the EMG increases. Note that there is a biphasic response in VL EMG (arrow heads). SOL activity is unchanged, except for the distinct transient increase just after the onset of the drop (arrow in C).

drop, applied approximately halfway through stance phase, produced an increase in EMG activity similar to that in figure 5.5B. The rapid, short duration increase in SOL activity is distinct (arrow in Fig. 5.5C), as well as the rapid prolonged increase in VL activity (in this case the VL EMG returned to control levels). A biphasic VL response to the sudden drop of the of hindquarters is apparent (arrow heads in Fig. 5.5C).

An important feature to note in Figure 5.5B and C is that the increase in EMG, which occurs during a rapid loading, precedes any detectable change in muscle force. This phenomenon indicates an afferent signal other than one related to muscle force is generating the initial change in muscle activity.

5.3.3. Removal of feedback by deafferentation

The experiments where the animals hindquarters were lifted or lowered indicated that changes in afferent feedback could selectively affect the level of activity in the quadriceps without affecting triceps surae activity. To further these results showing an independent regulation of muscles acting at the knee the consequence of selective deafferentation of the quadriceps muscle afferents was examined. The quadriceps afferents enter the spinal cord primarily through the L5-6 dorsal roots, whereas the triceps come in generally through the L7 and S1 roots. If the independent regulation of muscle activity were true, then selective deafferentation should preferentially reduce activity in the quadriceps (ie. according to which roots were cut).

All of the animals (n=4), with dorsal roots intact, produced periods of reliable decerebrate locomotion which was capable of supporting the animals weight. Three animals walked spontaneously after decerebration. One animal required constant stimulation of the MLR to produce any locomotion pattern.

Reduced EMG activity following partial deafferentation (L4 -L6)

Figure 5.6A compares the average EMG activity of VL in the intact state to that after transection of the L4-6 dorsal roots (a state that will be referred to as partial deafferentation) during periods of regular locomotor activity. The thin line is the average (n=25) EMG activity during walking with the dorsal roots intact. The thicker line is the average (n=25) VL EMG activity after transection of the L4 - L6 dorsal roots. There is

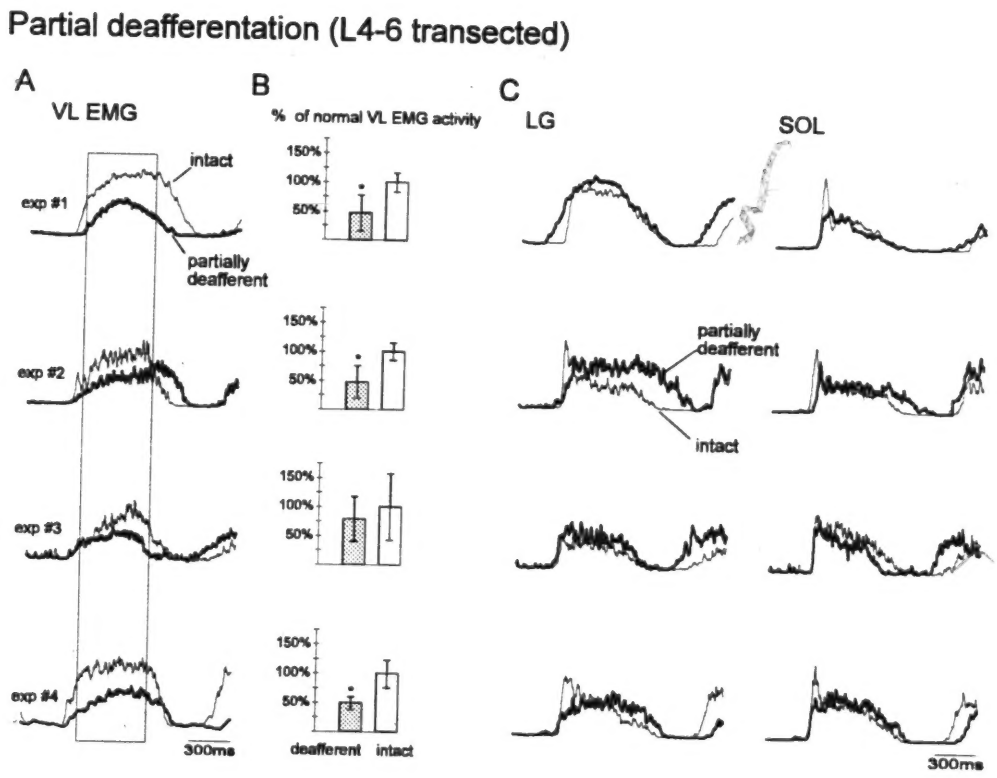

Figure 5.6. Averaged (n=25) rectified extensor EMG activity following L4 - L6 deafferentation as compared to intact muscle activity. A and C) The thick lines represent the deafferent EMG activity, while the thin lines indicate the intact level of activity. B) The average VL EMG amplitude has been calculated for a 500ms period (indicated by the shaded region in A) and is plotted in the histograms. The normal amplitude has been taken as 100% (open bars). The shaded bars indicate VL amplitude expressed as a precentage of the normal level when the animal is partially deafferented. Error bars indicate standard deviation. The asterisk indicates a significant reduction in EMG amplitude ($p<0.05$). Note that in all examples there was a distinct reduction in VL activity following partial deafferentation, whereas the amplitude of LG and SOL were relatively unaffected.

clearly a reduction in VL EMG activity following the loss of afferent input through the L4-L6 dorsal roots. The level of VL EMG activity following partial deafferentation has been plotted as the percentage of normal EMG activity in Figure 5.7B. The average VL EMG amplitude following partial deafferentation for each experiment was 47% ± 31 (standard deviation, SD), 47% ± 28, 79% ± 39 and 49% ± 11 that of the normal EMG activity seen during stance phase. All but the third value represent a statistically significant decrease in VL EMG. Interestingly, this one animal required constant stimulation of the MLR to produce locomotion. The data indicates that the loss of afferent feedback from the quadriceps muscle group produces a decrease in EMG activity to approximately 50% that of normal.

In general, partial deafferentation produced a slight trend towards an increase in the amplitude of triceps surae activity (Fig. 5.6C). In all experiments LG activity was increased (the 2nd and 3rd experiments representing significant increases). In two animals SOL activity increased slightly, whereas activity decreased in the other two. Thus the relatively selective removal of afferent feedback to the quadriceps reduced activity in those muscles, whereas extensor muscles acting at the ankle joint were not adversely affected by the loss of input.

Kinematic deficits after partial deafferentation

Given the reduction of quadriceps activity to approximately 50% of normal, deficits in the kinematics of walking were not unexpected. Following partial deafferentation one of the animals was not capable of supporting its body weight after partial deafferentation, in that the deafferented limb would collapse at the knee joint if no vertical support was provided. The other three animals following partial deafferentation were still capable of walking with independent weight support. However, there was a distinct increase in the yield of the knee joint observed at the beginning of the stance phase in the partially deafferented limb. It was qualitatively noted that the yielding of the knee joint was smallest in the animal which required MLR stimulation to produce walking [this animal also showed the least reduction in VL activity; exp#3 in Fig 5.6].

The kinematics of leg movements of one animal capable of weight support while intact and after partial deafferentation are illustrated is figure 5.7A. The lower position of the hindquarters during stance indicates that the deafferented limb was in a more flexed position than normal. This phenomenon is better illustrated by the angle plots (Fig. 5.7B). In the intact state there is a slight yield of the knee after the onset of stance phase as the weight of the animal is transferred onto the limb. In the partial deafferented state the yield at the knee is dramatically increased. The knee is becoming more flexed during the initial period of stance and only once the limb has moved out from directly underneath the animal, later in the stance phase, does the knee extend.

Full unilateral deafferentation

Successful unilateral deafferentation of the L7 and S1-2 dorsal roots was performed in 3 animals. This state of unilateral deafferentation, L4 - S2 transected, will be referred to as full deafferentation. Upon full deafferentation the locomotor rhythm became impaired for approximately 5-10 minutes. The deafferented limb was typically held rigid in extension, while the other three limbs continued to step on the treadmill. After the period of tonic extensor activity, a more regular locomotor rhythm eventually resumed in two animals. The third animal was at best only capable of making 4-5 poorly coordinated steps at a time, interrupted by periods of tonic extensor tonus.

At times the kinematics of the fully deafferented limb, in the two animals that walked, were not noticeably different from that observed during the partially deafferented state. The sag at the knee was still apparent, but an additional yielding at the ankle joint, which may have been anticipated to occur if afferent feedback was critical for producing sufficient muscle activity for support, was not observed. There were also periods of very erratic stepping, associated with periods of prolonged extensor tonus. During these periods flexion of the limb ranged between exaggerated flexion and limb placement, to a weak flexion of the limb under the body with the dorsum of the foot dragging behind.

The animal requiring MLR stimulation, after the initial period of extensor tonus and erratic stepping, eventually generated a stable and regular pattern of EMG activity.

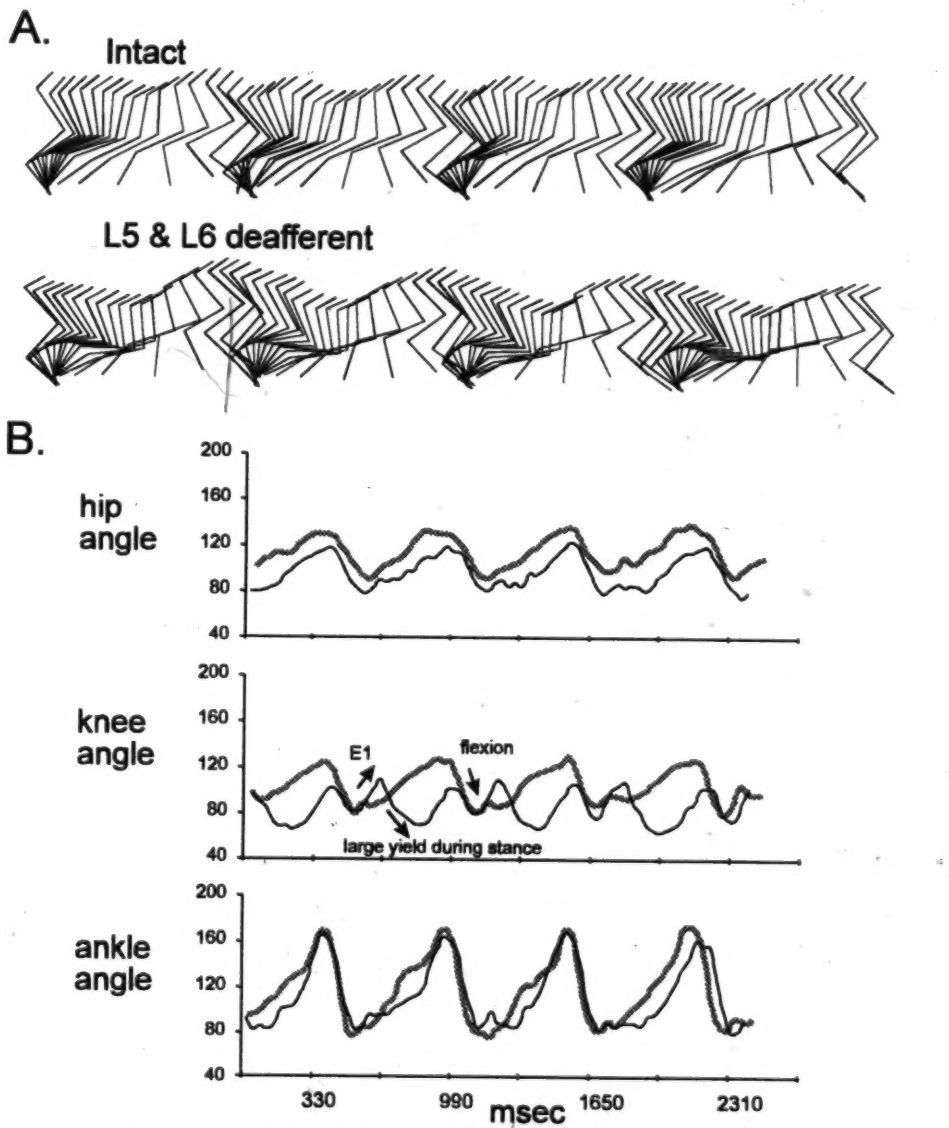

Figure 5.7. The kinematics of the left hindlimb of the decerebrate walking cat with dorsal roots intact and after transection of the 4th through 6th lumbar dorsal roots. A) Stick illustration of the limb over four step cycles. The interval between frames is 66 msec. Note that the deafferented limb sags during stance resulting in the lowering of the hindquarters. B) Joint angle plots comparing the movements at the hip, knee and ankle during intact and partially deafferented locomotion illustrated in the stick figures. Note that that there is a slight yielding of the knee after the E1 phase and that the yield is much greater at the knee joint after ground contact in the deafferent state. The hip and ankle joint angles are relatively unchanged.

The VL activity in the this animal, which did not show a significant reduction in amplitude following partial deafferentation (79%), was now significantly reduced to 42% of normal, intact activity. On the other hand, the extensor EMG activity of the other animal following full deafferentation was extremely variable as compared to the partial deafferent and intact states. During the more regular periods of full deafferented locomotion the average VL EMG amplitude showed some recovery of activity (57% of normal, as compared to 49% while partially deafferented). While stepping was erratic the EMG activity was extremely variable, not showing a significant change in amplitude as compared to normal levels of activity.

Full unilateral deafferentation did not affect the triceps surae muscles quite as anticipated. Given the distinct reduction in quadriceps activity following deafferentation of the L4-6 dorsal roots, it had been anticipated that the loss of afferent feedback from the triceps surae would produce a notable (ie. >50%) reduction in the EMG activity in those muscles. One of the animals that was capable of stepping after full deafferentation, showed the anticipated decrease in LG and SOL amplitude. The EMGs were reduced to 46% and 40% that of normal activity. In the second animal the reduction in EMG amplitude was not as apparent. LG activity was reduced to 78% of normal activity, but SOL activity remained as it had throughout the intact and partially deafferented states.

5.4. Discussion.

The role of afferent feedback in the production of locomotion has received considerable attention over the past two decades, with the emphasis on the control of the timing of the transition from stance to swing. However, upon the suppression of the corrective flexion response that normally occurs when a cat encounters a hole it was observed that the extensor muscle would remain active but at a very low level (see Fig. 3.2 in Chapter 3). This observation suggested that while the spinal neural circuitry was being supplied with sufficient feedback to control the timing of the locomotor activity, there was insufficient afferent feedback to generate the normal amplitude of the extensor EMG bursts.

Examination of the amplitude of extensor EMG activity when the limb was held in maintained extension in the hole revealed that the afferent feedback produced during stance phase with ground support generates approximately 80% of the extensor activity (see Figs. 5.1 and 5.2). The investigation by Severin (1970) indicated that approximately 50% of the triceps surae EMG activity during walking in the decerebrate cat is produced by muscle spindle afferent feedback. Yang et al. (1991) calculated a similar 30%-60% contribution of length sensitive afferents to SOL activity during walking in man. The additional 30% reduction in EMG amplitude observed in the present study can be accounted for by considering that all of the afferent feedback, rather than just muscle spindle feedback, was removed. In the study by Severin (1970) the reduction in EMG activity was produced by removing the gamma activation of the muscle spindles, while leaving other afferent feedback relatively unaffected (ie. tendon organ feedback and/or cutaneous afferents from the foot pad). As a result of the limb entering the hole the group Ia discharge, typically associated with the stretch of extensor muscles upon ground contact (Prochazka et al. 1989), would be absent. Along with the reduction in muscle spindle activity, the pressure sensitive cutaneous receptors on the foot pad are not activated, nor is any force produced in the extensor muscles as the limb extends freely when the limb encounters the hole. This general absence of all afferent feedback associated with the onset of stance could account for the larger reduction in extensor EMG observed as compared to the earlier studies.

The present data also suggest that the action of afferent feedback from the knee extensors is primarily restricted to other knee extensor muscles, and does not notably affect other extensor muscles of the hindlimb. It was demonstrated that following the removal of the afferent feedback to the quadriceps by transection of the L4-L6 dorsal roots the quadriceps activity was reduced by 50% without producing a change in the activity of triceps surae. Furthermore, during rapid lifting or lowering of the hindquarters quadriceps activity was decreased or increased, respectively, again without producing a change in the activity of triceps surae. If muscle afferent feedback was being utilized in a more distributed manner, ie. exciting extensors throughout the hindlimb, then altering quadriceps afferent input should have produced a concomitant change in ankle extensor

activity. Guertin et al. (1995) have noted a similar restriction of afferent feedback coming from the knee and hip extensors during fictive locomotion. Hip and knee extensor afferents typically facilitated electroneurogram (ENG) activity in nerves innervating muscles acting primarily across the hip and knee joints. Guertin and colleagues noted that knee extensor group I afferents often generated an inhibition of ankle extensor activity. Activation of group I afferents from semi-membranosus and anterior biceps during fictive locomotion did not produce any change in the amplitude of ankle extensor activity.

Whether a similar restriction of afferent feedback from the ankle extensors occurs can not be concluded from the results documented in the present experiment. The unreliable quadriceps EMG data collected during selective loading of the ankle extensors when the limb encountered the hole in the treadmill does not permit any firm conclusions. Nonetheless, evidence from other experiments suggests that ankle extensor afferents do not share a restricted pattern of influence, such as that observed for the knee extensors. Guertin et al. (1995) have shown that during fictive locomotion stimulation of group I afferents from MG, LG, or Plantaris were equally effective in increasing the amplitude of ankle, knee or hip extensor ENG activity. Similarly, Whelan et al. (1995a) have demonstrated that activation of the muscle spindle and tendon organ afferents from ankle extensors during walking in the decerebrate cat does produce a small increase in knee extensor activity.

5.4.1. Afferents regulating extensor burst activity

The data clearly indicates that afferent feedback is involved in the generation of extensor activity during walking. While the main objective of this study was not to identify the relative contribution of specific groups of afferents, the fact that resisting ankle extension when the limb entered the hole restored the level of muscle activity (see Fig. 5.3C) suggests that muscle afferents make a major contribution. This contribution could come from either the muscle spindles or the tendon organs in that the additional resistance generated by the wire could slow the rate of extension into the hole thereby

increasing activation of muscle spindle afferents and also increasing the discharge of tendon organs as the force is increased.

Previous studies have shown that during locomotion there are excitatory pathways from both the muscle spindle group Ia and tendon organ group Ib afferents onto extensor motoneurons. The most commonly known excitatory pathway from the muscle spindles is that of the monosynaptic reflex from the group Ia afferents (Eccles et al. 1957). Studies have also demonstrated that upon the onset of locomotion the non-reciprocal group I inhibition of synergistic motoneurons is attenuated (McCrea et al 1995). Concomitant with the reduction of group I inhibition, the onset of locomotion opens disynaptic (McCrea et al. 1995; Angel et al. 1996) and oligosynaptic (Gossard et al. 1994; Guertin et al. 1995) pathways from the extensor group Ia and group Ib afferents. Feedback from either of these pathways have been demonstrated to be capable of increasing extensor activity during locomotion (Conway et al 1987; Pearson and Collins 1993; Guertin et al. 1995).

The results of Severin (1970) and Yang et al. (1991) certainly indicate that the muscle spindle afferents contribute to the generation of EMG activity. The present results also implicate a contribution from the group Ia afferents. In figure 5.5B and 5.5C the increase in EMG activity occurs 20-30 ms after the onset of the ramp drop which rapidly loads the hindlimb, before there is a detectable change in force. This has been taken to indicate that afferent feedback from the muscle spindles is occurring before any input from the tendon organs. Furthermore, studies on the monosynaptic group Ia afferents reflex in the cat have shown that excitation of motoneurons is primarily restricted to homonymous and synergistic motoneurons innervating muscles acting at the same joint (Eccles et al. 1957; Edgley et al. 1986). The observation from the present study that afferent feedback is restricted to functionally synergistic muscles is consistent with this characteristic of the monosynaptic group Ia reflex.

The finding that the increase in muscle activity resulting from a sudden drop precedes a significant increase in force, does not rule out the possibility that tendon organ discharge also reinforces quadriceps activity. Once the muscle tension does increase the group Ib feedback may then act through excitatory interneurons to produce excitation of

the extensors (Gossard et al. 1994; Pearson and Collins 1993; McCrea et al. 1995). One observation suggesting that tendon organ feedback reinforces EMG activity is the reduction of VL activity when the animal was elevated during walking (Fig. 5.5). When the animal was allowed to carry its full weight the amount of force produced was approximately double that produced when the hindquarters were lifted slightly. The small changes in the joint angles indicate that differences in excursion of the muscles were relatively minor, potentially indicating only small changes in spindle activity. In this situation of assisting the weight support of the animal, the change in group Ib feedback is likely to be responsible for the change in EMG amplitude.

Thus during gait perturbations such as sudden loading (by dropping the hindquarters) it is postulated that the muscle spindles and the tendon organs are both activated and act in conjunction to regulate EMG activity. The initial stretch of the muscle elicits muscle spindle discharge which excites extensor motoneurons. The increased activity in the muscles produces an increase in force which generates additional Ib feedback to further reinforce the ongoing activity. This train of events is apparent in figure 5.5C. After the onset of drop, there is a short latency increase in knee extensor activity followed by a second component coinciding with the increase in force.

5.4.2. Functional role of afferent contribution to extensor activity

During locomotion the importance of peripheral feedback in the initiation of corrective responses to unexpected gait perturbations is apparent (Forssberg 1979; Gorassini et al. 1994). Peripheral afferent feedback allows corrective reactions to occur rapidly, enabling the animal to quickly adjust its gait and resume normal locomotion. However, as indicated by the reduction in extensor activity during maintained unloading of the hindquarters as compared to full weight support (Fig. 5.4), sensory feedback during locomotion is not limited in function to compensating for transient perturbations. The continuous regulation of EMG activity by afferent feedback would provide the locomotor circuitry with a large amount of flexibility, being able to automatically increase or decrease extensor activity to compensate for different external demands. For example, it has been shown that when walking up an inclined surface there is an increase

the extensor EMG activity, whereas when descending the EMGs are greatly reduced (Pierotti et al. 1989; Smith and Carlson-Kuhta 1995).

The importance of continuous afferent feedback is perhaps most dramatically illustrated during the extreme situation where almost all afferent feedback is removed to a particular muscle group. Following deafferentation of the L4-6 dorsal roots, removing most of the afferent input from the quadriceps, the amplitude of knee extensor activity was reduced resulting in the animal being incapable of producing normal support (Fig. 5.7). The extensive yield in the knee joint described in this paper is very similar to that documented previously by Grillner and Zangger (1984). Although not the focus of the investigation by Grillner and Zangger, they also attributed the increased yielding of the knee to the loss of reflex feedback that would normally reinforce muscle activity.

The conclusion that afferent feedback potentially contributes up to 80% of the extensor motor activity raises an interesting question as to what happens following full unilateral deafferentation, chronic spared root deafferentation or chronic peripheral nerve lesion experiments. Following full unilateral deafferentation, a reduction of extensor activity to only 20% that of normal would not have been surprising. However, the present results clearly indicate that this does not occur. The fact that sufficient levels of extensor activity were generated to provide weight support, iregardless of the extra yielding of the limb, suggests a fairly rapid reorganization of the spinal circuitry. A significant portion of this may be due to substituting descending drive for the lost afferent feedback. This has been suggested to be the case following chronic unilateral deafferentation (Goldberger and Murray 1988). The qualitative observation that the locomotor rhythm following full unilateral deafferentation in the present study was more stable in the preparation with MLR stimulation is consistent with this hypothesis.

Deafferented cats with a single spared dorsal root as compared to full unilateral deafferentation have been shown to be more capable of recovering a near normal pattern of walking. The fact that this recovery persists if supraspinal input is subsequently removed (by spinal hemisection ipsilateral to the deafferentation) indicates that this recovery is a function of some adaptation at the spinal level (Goldberger 1983). It has been hypothesized that following spared root deafferentation that the remaining afferent

input is used to a greater extent, thus compensating for the loss (Goldberger and Murray 1988). Recently, Whelan et al. (1995b) have shown that following lesion of an extensor nerve the synaptic efficacy of group I afferents from functionally synergistic muscles rapidly increases. Apparently, the nervous system is capable of recalibrating the afferent feedback system so that following alterations to the amount of afferent feedback produced the reflex response is still functionally appropriate. How this is achieved still requires further investigation.

5.5. References.

Angel, M.J., Guertin, P., Jim,nez, I. and McCrea. D.A. Group I extensor afferents evoke disynaptic EPSPs in cat hindlimb extensor motoneurones during fictive locomotion. *J. Physiol.* 494:851-861, 1996.

Conway, B. A., Hultborn, H., and Kiehn, O. Proprioceptive input resets central locomotor rhythm in the spinal cat. *Exp. Brain Res.* 68:643-656, 1987.

Duysens, J. and Pearson, K. G. Inhibition of flexor burst generation by loading ankle extensor muscles in walking cats. *Brain Res.* 187:321-332, 1980.

Edgley, S. A., Jankowska, E., and McCrea, D. A. The heteronymous monosynaptic actions of triceps surae group Ia afferents on hip and knee extensor motoneurones in the cat. *Exp. Brain Res.* 61:443-446, 1986.

Eccles, J. C., Eccles, R. M, and Lundberg, A. The convergence of monosynaptic excitatory afferents on to many different species of alpha motoneurones. *J. Physiol.* 137:22-50, 1957.

Forssberg, H. Stumbling corrective reaction: a phase dependent compensatory reaction during locomotion. *J. Neurophysiol.* 42:936-953, 1979.

Goldberger, M. E. Recovery of accurate limb movements after deafferentation in cats. In: *Spinal Cord Reconstruction*, edited by C. C. Kao, R. P. Bunge and P. J. Reier. New York: Raven Press, 1983, p. 455-463.

Goldberger, M. E. and Murray, M. Patterns of sprouting and implication for recovery of function. In: *Advances in Neurobiology*. Vol.47: Functional recovery in neurological diesease, edited by S. G. Waxman. New York: Raven Press, 1988, p. 361-385.

Gorassini, M. A., Prochazka, A. K., Hiebert, G. W., and Gauthier, M. J. A. Corrective responses to loss of ground support during walking I. Intact cats. *J. Neurophysiol.* 71:603-610, 1994.

Gossard, J. -P. , Brownstone, R. M. , Barajon, I. , and Hultborn, H. Transmission in a locomotor-related group Ib pathway from hindlimb extensor muscles in the cat. *Exp. Brain Res.* 98:213-228, 1994.

Grillner, S. and Zangger, P. The effect of dorsal root transection on the efferent motor pattern in the cat's hindlimb during locomotion. *Acta. Physiol. Scand.* 120:393-405, 1984.

Guertin, P., Angel, M., Perreault, M. -C., and McCrea, D. A. Ankle extensor group I afferents excite extensors throughout the hindlimb during MLR-evoked fictive locomotion in the cat. *J. Physiol.* 487(1):197-210, 1995.

Hagbarth, K., Kunesch, E. J., Nordin, M., Schmidt, R., and Wallin, E. U. Gamma loop contributing to maximal voluntary contractions in man. *J. Physiol.* 380:575-591, 1986.

Laporte, Y. and Lloyd, D. P. C. Nature and significance of the reflex connections established by large afferent fibers of muscular origin. *Am. J. Physiol.* 169:609-621, 1952.

Macefield, V. G., Gandevia, S. C., Bigland-Rithchie, B., Gorman, R. B., and Burke, D. The firing of human motoneurones voluntarily activated in the absence of muscle afferent feedback. *J. Physiol.* 471:429-443, 1993.

McCrea, D. A., Shefchyk, S. J., Stephens, M. J., and Pearson, K. G. Disynaptic group I excitation of synergistic ankle extensor motoneurones during fictive locomotion. *J. Physiol.* 487(2):527-540, 1995.

Pearson, K. G. and Collins, D. F. Reversal of the influence of group Ib afferents from plantaris on activity in medial gastrocnemius muscle during locomotor activity. *J. Neurophysiol.* 70:1009-1017, 1993.

Pierotti, D. J. , Roy, R. R. , Gregor, R. J. , and Edgerton, V. R. Electromyographic activity of cat hindlimb flexors and extensors during locomotion at varying speeds and inclines. *Brain Res.* 481:57-66, 1989.

Prochazka, A. K. Chronic Techniques for Studying Neurophysiology of Movement in Cats. In: *Methods for Neuronal Recording in Conscious Animals*, edited by R. Lemon. New York: John Wiley, 1984, p. 113-128.

Prochazka, A., Trend, P. St. J., Hulliger, M., and Vincent, S. Ensemble proprioceptive activity in the cat step cycle: towards a representative look-up chart. *Prog. Brain Res.* 80:61-74, 1989.

Severin, F. V., Orlovsky, G. N., and Shik, M. L. Work of the muscle receptors during controlled locomotion. *Biophysics.* 12:502-511, 1967.

Severin, F. V. The role of the gamma motor system in the activation of the extensor alpha motor neurones during controlled locomotion. *Biophysics.* 15:1096-1102, 1970.

Sherrington, C. S. Flexion-reflex of the limb, crossed extension-reflex, and reflex stepping and standing. *J. Physiol.* 40:28-121, 1910.

Shik, M. L., Severin, F. V., and Orlovsky, G. N. Control of walking and running by means of electrical stimulation of the mid-brain. *Biophysics* 11:756-765, 1966.

Smith, J. L. and Carlson-Kuhta, P. Unexpected motor patterns for hindlimb muscles during slope walking in the cat. *J. Neurophysiol.* 74(5):2211-2215, 1995.

Whelan, P.J. Function of extensor group I afferents feedback in the control of locomotion. Ph.D. Thesis. University of Alberta. 1996

Whelan, P. J., Hiebert, G. W., and Pearson, K. G. Stimulation of the group I extensor afferents prolongs the stance phase in walking cats. *Exp. Brain Res.* 103:20-30, 1995a.

Whelan, P. J., Hiebert, G. W., and Pearson, K. G. Plasticity of the extensor group I pathway controlling the stance to swing transition in the cat. *J. Neurophysiol.* 74:2782-2787, 1995b.

Yang, J. F. , Stein, R. B. , and James, K. B. Contribution of peripheral afferents to the activation of the soleus muscle during walking in humans. *Exp. Brain Res.* 87:679-687, 1991.

CHAPTER 6.

GENERAL DISCUSSION

One role of peripheral sensory feedback during locomotion is to regulate the stepping pattern so that it meets the demands imposed by the changing external environment. A particularly clear example of this function is the "stumbling corrective reaction" documented by Forssberg (1979). A stimulus to the dorsum of the foot, which potentially represents an object that could "trip" the animal, results in increased flexion during swing phase (lifting the foot over the obstacle) or increased extensor activity during stance (resisting the obstacle). This adaptation of the step cycle allows the animal to continue its progress with minimum disturbance to the locomotor pattern.

Chapter 2 of this thesis characterized another type of reaction to an obstacle that might be encountered in the environment, that of a hole in the supportive surface. When a limb encounters the absence of ground support the limb is extended into the hole, but is then rapidly flexed out and replaced in front of the obstacle. At the same time there is an increase in the activity of the contralateral extensors and a prolonged duration of stance phase. Like the stumbling corrective reaction this corrective flexion reaction is extremely efficient, with the coordinated action of both limbs allowing the animal to continue progression with minimum disturbance. Using this characteristic "Foot-in-Hole" response as a model and as a tool, this thesis investigated the contribution of afferent feedback to the generation of muscle activity during walking in the cat.

6.1. Afferent control of the locomotor rhythm.

The results of **Chapter 2** revealed that while the corrective flexion response was facilitated by supraspinal structures the neural circuitry responsible for generating this response was located in the lumbar region of the spinal cord. It was concluded that afferent feedback was responsible for the initiation of the flexion response. Two potential sources of the afferent feedback were considered, one coming from afferents signaling

extension of the hip and the other from muscle afferent feedback signaling loading of the limb. At the time Chapter 2 was completed, particular emphasis was placed on the potential role of the absence of tendon organ group Ib feedback from the limb entering the hole. This emphasis stemmed from the observations that activation of extensor Ib afferents could reset the locomotor rhythm from flexor to extensor activity (Conway et al. 1987), entrain the locomotor rhythm (Pearson et al. 1992), and facilitate extensor activity during locomotion (Pearson and Collins 1993). These observations indicated that group Ib feedback, which is inhibitory in the quiescent animal, acts to reinforce extensor activity during locomotion. Therefore it was hypothesized that the absence of feedback from the tendon organs from the limb entering the hole resulted in extensor activity not being reinforced and hence permitted the flexors to become active (see Fig 2.10).

Chapter 3 tested the hypothesis proposed in Chapter 2 by activating extensor group I afferents as the limb entered the hole. It was demonstrated that a necessary feature for the initiation of a flexion response out the hole was the absence of extensor group I feedback. Again, based on the past evidence that group Ib feedback, and not group Ia feedback, could alter the locomotor rhythm (Conway et al. 1987; Pearson et al. 1992; Pearson and Collins 1993), it was concluded that the group Ib afferents were responsible for suppressing the flexion response out of the hole. This conclusion was further reinforced by new evidence that demonstrated the state dependent reversal of group I afferent inhibition to excitation upon the onset of locomotion (Gossard et al. 1994).

Since the completion of the experiments documented in Chapter 3, extensor muscle spindle group Ia afferents have also been shown to have excitatory disynaptic (McCrea et al. 1995) and oligosynaptic connections (Guertin et al. 1995a) to extensor motoneurons. Contrary to that documented previously by Conway et al. (1987), these recent investigations demonstrated that activation of the muscle spindles alone are capable of prolonging extensor activity and resetting the locomotor rhythm. Thus, the reduced level of extensor muscle spindle discharge when a limb encounters a hole [no stretch of the extensor muscles resulting from the yielding of the limb following normal

ground contact (Prochazka et al. 1989)] may act in a manner similar to that of the group Ib afferents by not reinforcing stance phase activity.

The result from Chapter 3 indicated that feedback from extensor group I afferents could precisely regulate the timing of the flexion response out of the hole (see Fig. 3.3). This observation has since been extended to show that group I feedback can regulate the duration of stance phase activity during normal walking in the decerebrate cat. Whelan et al. (1995) demonstrated that electrical stimulation of group I extensor afferents during decerebrate locomotion is capable of distinctly prolonging stance phase duration. Very similar to that observed during the suppression of the corrective flexion response, Whelan and colleagues observed that the onset of swing phase usually occurred only after the cessation of the afferent stimulation. This observation coupled with that presented in Chapter 3, provide evidence that an afferent signal indicating unloading of the limb is a required component before the transition from stance to swing can occur.

Chapter 4 addressed the potential contribution of afferents indicating extension of the limb in the initiation of the corrective flexion response to the absence of ground support. Simulating extension of the hip or ankle, by stretching the IP muscle or electrically stimulating afferents from EDL or TA, promoted the initiation of a flexion response out of the hole. Furthermore, activation of the group I flexor muscle afferents during stance phase activity shortened the duration of extensor activity, leading to an earlier onset of flexor activity.

The results of Chapter 4 clarify and extend the earlier observations made by other investigators. The studies of Grillner and colleagues (Grillner and Rossignol 1978; Andersson and Grillner 1981, 1983) revealed that the afferent signal produced by hip extension was important in the generation of the stance-to-swing transition. During fictive locomotion in the cat holding the limb in a static flexed or extended position distinctly alters the locomotor rhythm (Pearson and Rossignol 1991; Rossignol et al. 1993). When the leg is held flexed the rhythm is dominated by extensor activity. Extension of the limb causes the flexor activity to become more robust, and the extensor activity to be reduced. However, the identity or origin of the particular afferents

responsible for this effect were not elucidated. In 1994 Kriellaars et al. demonstrated that the entrainment of the fictive locomotor rhythm by sinusoidal flexion/extension of the hip was not dependant on afferent feedback from receptors located in the hip joint capsule. While the results from Kriellaars et al. (1994) implicated the musculature acting around the hip joint as providing an afferent signal which generates the onset of flexor activity, their results did not provide an indication as to the relative contribution of individual muscles. The results from Chapter 4 indicated that the muscle spindle afferent discharge produced by stretch of the hip flexor iliopsoas muscle is one potential source of this afferent signal. The experiments documented in Chapter 4 furthered the ideas generated in the previous studies by demonstrating that afferent feedback from muscles other than those acting at the hip joint could function to facilitate the onset of swing phase. Stretch of the ankle flexor muscles, simulating extension of the ankle joint, was shown to also advance the onset of swing phase activity. Thus, this study demonstrated that the afferent feedback signaling the lengthening of flexor muscles is involved in the onset of normal swing phase activity.

One interesting finding of Chapter 4 was that the afferent feedback from the flexor muscles of an immobilized limb was sufficient to entrain the locomotor rhythm of a decerebrate cat that had the other three limbs stepping on a moving treadmill. To date most studies on the entrainment of the locomotor rhythm have been performed on either paralyzed fictively locomoting animals (Andersson and Grillner 1983; Conway et al. 1987; Kriellaars et al. 1994) or extensively deinnervated spinal preparations (Pearson et al. 1992) where the applied stimulation is the only peripheral timing cue present. The fact that the timing of the locomotor rhythm was dictated by the afferent feedback from the flexor muscles, despite the presence of the menagerie of peripheral signals produced by overground locomotion, indicates that the flexor muscle afferents generate a powerful signal which is capable of regulating the locomotor rhythm.

An unresolved issue from Chapter 4 is the exact mechanism by which the flexor muscle afferents contribute to the resetting of the locomotor rhythm. In Chapter 4 it was proposed that the spinal circuitry generating extensor activity was inhibited by the flexor muscle afferents, thus disinhibiting the flexor half-centre. Recently Guertin et al. (1995b)

have reported that during resetting of the fictive locomotor rhythm by stimulation of extensor group I afferents that a simultaneous short latency (~2ms) hyperpolarization of flexor motoneurons occurs concomitant with depolarization of extensor motoneurons (Guertin et al. 1995b). It would be interesting to explore whether or not the flexor muscle afferents produce a similar depolarization of synergistic and hyperpolarization of antagonistic motoneurons, potentially affecting both flexor and extensor half-centres simultaneously. However, before undertaking such an experiment the incongruencies between the results reported in Chapter 4 and those of Perreault et al. (1995) would have to be addressed. Perreault and colleagues reported that stimulation of group II afferents from TA, sartorius or posterior biceps during flexor activity served to reset the fictive locomotor rhythm to extension. The results from Chapter 4 indicated that stretch of IP during swing phase activity produced no change in the locomotor rhythm, while electrical activation of EDL or TA group II afferents could prolong flexor activity. Activation of afferents from flexor muscles during extensor activity was shown by Perreault et al. (1995) to produce weak effects on the locomotor timing and to occasionally increase activity in the hip extensors. These results directly conflict with the early termination of extensor activity as result of flexor muscle afferent stimulation documented in Chapter 4. Additional experiments in the fictive animal examining the reflex effect of afferent feedback from other pure flexor muscles such as IP, rather than bifunctional muscles such as sartorius or posterior biceps, would be the first order of investigation. It would also be beneficial to examine the effect of activating afferents from sartorius or ST during walking in the decerebrate cat.

Unloading and limb extension are required for the corrective flexion response

Together the results from Chapter 3 and Chapter 4 begin to identify the afferent feedback which initiates the corrective flexion reaction associated with the absence of ground support. As the limb enters the hole the lack of extensor group I feedback fails to reinforce the spinal circuitry producing extensor activity. The rapid extension of the limb into the hole also activates the flexor muscle spindle afferents which excite the neural mechanism involved in the generation of flexor activity. Together the interaction of these

afferent signals generate the initiation of the flexion reaction. The present data also indicate that both the absence of loading and the extension of the limb are required for triggering flexion of the limb.

While an absence of load on the extensor muscles is a requirement in the production of a corrective flexion response out of the hole (Chapter 3), there are indications that unloading of the muscle alone is insufficient to produce a flexion response. In Chapter 4, the insertions of the extensor muscle on the fixed limb were cut, allowing these muscles to contract freely and produce very little force. If the absence of an afferent signal indicating loading of the limb were the critical signal in the flexion response then it would follow that a maintained unloading should result in rapid cyclic activity in the flexors. For example, in the decerebrate quadrupedal preparation a repetitive initiation of flexor EMG activity (at approximately 2.5 Hz) occurs if during a "Foot-in-Hole" reaction the limb is re-extended into the hole instead of being placed on the treadmill belt, i.e. a second corrective flexion response is initiated (unpublished personal observations). However, in the fixed limb preparation, when the extensor muscles are not loaded (i.e. patella tendon is cut, calcaneum detached, and hip extensors denervated) a repetitive discharge of flexor muscle activity was not observed. These observations indicate that some other afferent signal is required for the initiation of a corrective flexion response.

In the same sense that unloading alone is not sufficient, the afferent signal indicating extension of the limb by itself is also insufficient for eliciting flexion of the limb. In the study by Whelan et al. (1995) stimulation of extensor group I afferents was observed to prolong the duration of stance phase. The prolongation of stance phase could occur for an extended period of time (ie. 2000ms) with the limb held in an extended position (see Fig 11 in Whelan 1996b). In this situation it is clear that the afferent signal generated by extension of the limb is not sufficient to overcome the simulated loading of the limb by activation of the extensor group I afferents.

6.2. Afferent regulation of extensor activity.

In Chapter 2 a distinct increase in the contralateral extensor activity was observed as the ipsilateral limb entered the hole (Fig. 2.9). Originally, the increase in EMG amplitude was attributed to the increased load carried by the limb as signaled by the tendon organs. As shown in Chapter 5 mimicking the sudden loading of the supporting limb by rapidly lowering the animals hindquarters produced a very similar profile of activity in the knee extensors (Fig. 5.5B). However, the fact that the EMG amplitude increased before a noticeable change in force indicated that force sensitive afferents alone were not responsible for the facilitated activity. It was proposed that the sudden loading of the limb generates a stretch of the muscles which activates the group Ia afferents. The muscle spindles evoke a stretch reflex which subsequently activates the tendon organs to further reinforce the EMG activity. Thus, muscle spindles and tendon organ feedback act together to compensate for the sudden loading of the limb.

The observation from Chapter 3 that there was low activity in the extensor muscles during suppression of the corrective flexion reaction, indicated that peripheral afferent feedback not only serves to compensate for sudden perturbations but is required for the production of the normal level of EMG activity during locomotion. In Chapter 5 examination of the extensor EMG amplitude when the foot entered the hole in the treadmill revealed that up to 80% of the EMG activity observed during walking in the decerebrate cat is produced by afferent feedback. This result furthered previous estimates regarding the afferent contribution to locomotor EMG activity. In 1970, Severin demonstrated that group Ia feedback produced almost 50% of triceps surae muscle activity during decerebrate locomotion. The greater value of 80% represents the contribution of the total afferent feedback generated as a result of the limb pushing against the ground during stance phase.

6.3. Integrating the potential reflex pathways involved in the regulation of extensor activity.

An unresolved issue is the relative contribution of the monosynaptic, disynaptic and oligosynaptic pathways in the generation of extensor EMG activity. Based on the

ability of extensor group I afferent feedback to reset (Conway et al. 1987) and entrain (Pearson et al. 1991) the locomotor rhythm in acute spinal preparations, the interneurons interposed in the oligosynaptic pathway have been proposed to be part of the spinal circuitry which generates extensor activity during locomotion (Gossard et al. 1994). It is this pathway which is postulated to suppress the corrective flexion response (Chapter 3) and prolong stance phase activity (Whelan et al. 1995) during activation of extensor group I afferents. As part of the central locomotor circuitry, afferent feedback acting through the oligosynaptic pathway could be anticipated to produce a general excitation of extensor motoneurons. This prediction is supported by evidence in fictive spinal preparations (Pearson and Collins 1993), where the group I disynaptic pathway is not open and hence not capable of contributing any excitation (McCrea et al. 1995). In acute spinal preparations, activation of quadriceps extensor group I afferents has been shown to generate excitation of more distant heteronymous motoneurons (for examples see Fig 1C from Conway et al. 1987; Figs. 1C, and 3A in Gossard et al. 1994). These results suggest that muscle afferent feedback can act in a global manner on the extensor muscles throughout the hindlimb. However the results from Chapter 5 indicated that knee extensor afferent feedback primarily reinforces functionally synergistic motoneurons.

Afferent feedback acting through the locomotor dependent disynaptic pathway produces depolarization across a more restricted group of extensor motoneurons. Angel et al. (1996) have shown that group I afferents from hip extensors fail to produce depolarization of ankle extensor motoneurons, whereas functionally synergistic motoneurons are excited. Furthermore, knee extensor afferents have been shown to facilitate activity in knee (Whelan et al. 1995) and hip (Guertin et al. 1995a) extensors, while producing inhibition of ankle extensor activity (Whelan et al. 1995; Guertin et al. 1995a). These earlier results showing a more restricted convergence of the action of the knee and hip extensor muscle afferents are in better agreement with the observations made in Chapter 5, where a distinct change in quadriceps EMG activity was not paralleled by a similar change in triceps surae.

Ankle extensor afferents do not appear to follow a restricted pattern of excitatory action in the decerebrate preparation. McCrea and colleagues (Guertin et al. 1995a;

Angel et al. 1996) have shown that as well as exciting synergistic motoneurons, activation of ankle group I afferents during fictive locomotion can excite motoneurons of the knee and hip extensors. In decerebrate cats walking on a moving treadmill, stimulation of ankle extensor afferents also produces an excitation of quadriceps activity (Whelan et al. 1995), although the intensity of this excitation has recently been questioned (Whelan 1996a).

Why fictive spinal preparations demonstrate a pronounced general facilitation of extensor activity following stimulation of extensor muscle afferents, whereas decerebrate preparations show a more restricted pattern of afferent connectivity is a matter of speculation. A possible explanation is that supraspinal structures not present in the acute spinal preparation may act to modulate the afferent transmission. Stimulation of the MLR in the decerebrate cat has been shown to reduce the ability of extensor group I afferents to prolong the stance phase duration (Whelan 1996a). Angel et al. (1996) proposed that there may be two subpopulations of excitatory interneurons receiving input from the extensor group I afferents; one group projecting to a broad range of extensor motoneurons and the other having no projections to ankle extensor motoneurons. Supraspinal structures may act to selectively reduce transmission through these pools of interneurons.

The amalgamation of data from other laboratories permits some speculation regarding which pathways are primarily responsible for mediating the afferent regulation of extensor EMG duration and amplitude. An attractive approach when surmising the neural circuitry involved in the afferent regulation of locomotor activity is to assign particular functions to the various pathways. The monosynaptic and disynaptic reflex pathways act to ensure that the output of muscles acting at particular joints is adequate to meet the motor demands of the moment. In this sense the role of the monosynaptic group Ia and disynaptic group I pathways is to regulate the amplitude of the extensor activity. Central mechanisms, whether spinal or supraspinal in origin, would act to restrict the divergence of the afferent feedback so that only select muscle groups are reinforced. This type of organization would allow the locomotor system to compensate for perturbations acting at a single joint without changing the entire locomotor rhythm.

As discussed throughout this thesis there is evidence indicating that the extensor group I oligosynaptic pathway contains interneurons which are part of the central circuitry generating the extensor bursts during locomotion. Extensor group I afferent feedback acting on the extensor half-centre through the oligosynaptic pathway would primarily influence the timing or duration of extensor activity [see Introduction for description of Brown's (1911) and Lundberg's (1981) "half-centre" model for locomotion]. New evidence also suggests that the excitation of the extensor half-centre is accompanied by inhibition of the flexor half-centre (Guertin et al. 1995b). By maintaining or reinforcing activity in the extensor half-centre, extensor afferent feedback would serve to ensure continued stance phase activity for as long as the limb was loaded. Acting in tandem with the extensor oligosynaptic afferent pathway would be the flexor muscle afferents. As discussed in Chapter 4, flexor afferent feedback primarily affects the timing of the locomotor rhythm by potentially inhibiting activity in the extensor half-centre, and/or exciting the flexor half-centre. Thus, there are a number of systems which interact to guarantee that locomotion continues in the smoothest manner possible. One point that must be kept in mind is that during other forms of locomotion the usefulness of afferent cues identified in this thesis (loading of extensor muscles or extension of the hip) may not be as great. For example, when a cat walks backwards the transition from stance to swing occurs when the hip reaches a flexed rather than extended position (Buford et al. 1990; Buford and Smith 1990). Also, when a cat walks down a steep slope the extensor muscles are weakly active, potentially generating a lower level of extensor afferent feedback, and there is prominent hip and ankle flexor activity which presumably functions to slow the extension of the limb (Smith and Carlson-Kuhta 1995). Based on these examples it is apparent that the relevance given to a particular afferent cue by the nervous system during level forward locomotion may be altered during different locomotor tasks. How the sensorimotor rules are adapted to cope with new locomotor demands remains a interesting field for investigation.

6.4. Closing Statements.

The work presented in this thesis characterizes a specific corrective reaction which occurs when a walking cat encounters a hole in the supportive surface. A number of potential sources of afferent feedback were postulated to be involved in the generation of the flexion response, and in the production of the increased contralateral extensor activity which provides maintained support of the animals hindquarters. Afferent feedback from the flexor muscles, indicating the extension of the limb, was shown to influence the timing of the corrective flexion response. Furthering this observation, flexor afferent feedback was demonstrated to be involved in generating the transition from stance to swing during normal locomotion. The absence of extensor muscle group Ia and group Ib afferent feedback from the limb entering the hole was shown to be necessary for a flexion reaction to be initiated. In subsequent studies a large contribution of the extensor group I afferent feedback to the timing and amplitude of extensor activity has been demonstrated. Throughout this thesis, the corrective reaction to the absence of ground support has proven to be a useful "tool" for studying the role of afferent feedback during locomotion.

The usefulness of this characteristic corrective flexion response has not yet been exhausted. One potential use of this tool would be to assess the level or degree of recovery following a lesion to the nervous system. Goldberger (1988) while studying partial deafferentation tested the locomotor abilities of his cats by challenging them with runways of varying difficulty. The absence of ground support during walking could be used as a similar type of challenge, which may further reveal the extent to which the nervous system has recovered. For example, following selective lesion to various regions of the spinal cord a "normal" appearing locomotor pattern may recover, but the adaptive capabilities of the animal to locomotor challenges remains in question (see Appendix I). Further examination of the recovery of locomotor abilities following spinal cord injury is a tempting area for future research.

6.5. References.

Andersson, O. and Grillner, S. Peripheral control of the cat's step cycle: I. Phase dependent effects of ramp-movements of the hip during `fictive locomotion'. *Acta Physiol. Scand.* 113:89-101, 1981.

Andersson, O. and Grillner, S. Peripheral control of the cat's step cycle: II. Entrainment of the central pattern generators for locomotion be sinusoidal hip movements during `fictive locomotion'. *Acta Physiol. Scand.* 118:229-239, 1983.

Angel, M. J., Guertin, P., Jimenez, I., and McCrea, D. A. Group I extensor afferents evoke disynaptic EPSPs in cat hindlimb extensor motoneurons during fictive locomotion. *J. Physiol.* 494:851-861, 1996.

Brown, T. G. The intrinsic factors in the act of progression in the mammal. *Proc. R. Soc.* 84:308-319, 1911.

Buford, J. A, Zernicke, R. F., and Smith, J. L. Adaptive control of backward quadrupedal walking. I. Posture and hindlimb kinematics. *J. Neurophysiol.* 64:745-755, 1990.

Buford, J. A and Smith, J. L. Adaptive control for backward quadrupedal walking II. Hindlimb muscle synergies. *J. Neurophysiol.* 64:756-766, 1990.

Conway, B. A., Hultborn, H., and Kiehn, O. Proprioceptive input resets central locomotor rhythm in the spinal cat. *Exp. Brain Res.* 68:643-656, 1987.

Forssberg, H. Stumbling corrective reaction: a phase dependent compensatory reaction during locomotion. *J. Neurophysiol.* 42:936-953, 1979.

Goldberger, M. E. Spared-root deafferentation of a cat's hindlimb: hierarchical regulation of pathways mediating recovery of motor behaviour. *Exp. Brain Res.* 73:329-342, 1988.

Gossard, J. -P. , Brownstone, R. M. , Barajon, I. , and Hultborn, H. Transmission in a locomotor-related group Ib pathway from hindlimb extensor muscles in the cat. *Exp. Brain Res.* 98:213-228, 1994.

Guertin, P., Angel, M. J., Perreault, M. -C., and McCrea, D. A. Ankle extensor group I afferents excite extensors throughout the hindlimb during MLR-evoked fictive locomotion in the cat. *J. Physiol.* 487(1):197-210, 1995a.

Guertin, P., Perreault, M. -C., and McCrea, D. A. Organization of the CPG revealed by perturbation of the step cycle evoked by group I afferents. *Soc. Neurosci. Abst.* 21:277.8, 1995b.

Grillner, S. and Rossignol, S. On the initiation of the swing phase of locomotion in chronic spinal cats. *Brain Res.* 146:269-277, 1978.

Kriellaars, D. J., Brownstone, R. M., Noga, B. R., and Jordan, L. M. Mechanical entrainment of fictive locomotion in the decerebrate cat. *J. Neurophysiol.* 71:2074-2086, 1994.

Lundberg, A. Half-centres revisited. In: *Regulatory Functions of the CNS. Motion and Organizational Principles*, edited by J. Szentagothai, M. Palkovits and J. Jamori. Pergamon Press, p. 155-167, 1980.

McCrea, D. A., Shefchyk, S. J., Stephens, M. J., and Pearson, K. G. Disynaptic group I excitation of synergistic ankle extensor motoneurones during fictive locomotion. *J. Physiol.* 487(2):527-540, 1995.

Pearson, K. G. and Collins, D. F. Reversal of the influence of group Ib afferents from plantaris on activity in medial gastrocnemius muscle during locomotor activity. *J. Neurophysiol.* 70:1009-1017, 1993.

Pearson, K. G. and Rossignol, S. Fictive motor patterns in chronic spinal cats. *J. Neurophysiol.* 66:1874-1887, 1991.

Pearson, K. G., Ramirez, J. M., and Jiang, W. Entrainment of the locomotor rhythm by group Ib afferents from ankle extensor muscles in spinal cats. *Exp. Brain Res.* 90:557-566, 1992.

Perreault, M. -C., Angel, M., Guertin, P., and McCrea, D. A. Stimulation of hindlimb flexor group II afferents during fictive locomotion. *J. Physiol.* 487(1):211-220, 1995.

Rossignol, S., Saltiel, P., Perreault, M. -C., Drew, T., Pearson, K. G., and Belanger, M. Intralimb and interlimb coordination in the cat during real and fictive rhythmic motor programs. *Seminars Neurosci.* 5:67-75, 1993.

Severin, F. V. The role of the gamma motor system in the activation of the extensor alpha motor neurones during controlled locomotion. *Biophysics* 15:1096-1102, 1970.

Smith, J. L. and Carlson-Kuhta, P. Unexpected motor patterns for hindlimb muscles during slope walking in the cat. *J. Neurophysiol.* 74:2211-2215, 1995.

Whelan, P.J. Function of extensor group I afferents feedback in the control of locomotion. Ph.D. Thesis. University of Alberta. 1996a.

Whelan, P. J. Control of locomotion in the decerebrate cat. *Progress in Neurobiology.* in press:1996b.

Whelan, P. J., Hiebert, G. W., and Pearson, K. G. Stimulation of the group I extensor afferents prolongs the stance phase in walking cats. *Exp. Brain Res.* 103:20-30, 1995.

Appendix I.

Contribution of ascending and descending spinal pathways in the generation of the corrective flexion response.

In Chapter 2 a corrective reaction observed in bipedally walking cats in response to a hole in the supportive surface was examined. The differences in the corrective flexion response in intact and chronic spinal cats indicated that 1) peripheral afferent feedback were involved in initiating the flexion reaction, and 2) supraspinal structures facilitated the initiation of flexion response in the intact animal. Thus, after completion of the research documented in Chapter 2 there were two potential directions for future research. One was to further examine the role of afferent feedback. The other was to examine which supraspinal structures were facilitating the onset of the flexion response in intact animals.

This Appendix documents preliminary data investigating the contribution of various ascending and descending spinal pathways to generation of the corrective flexion response observed in chronic cats. The primary objective of this study was to examine the latency of the corrective flexion reaction following chronic unilateral lesion of the dorsal column or dorsolateral funiculus of the spinal cord.

Four animal were used in this study. The methodology used in these experiments is very similar to that described in Chapter 2, therefore only a brief account will be given. Each animal was trained to walk bipedally on a motor driven treadmill (0.35m/sec). Once trained to produce reliable bipedally walking, the treadmill belt was replaced with one with a hole (10 cm x 30 cm) on the left side. As in Chapter 2 this resulted in the left hind leg occasionally stepping into the hole.

During an aseptic surgical procedure the spinal cord was exposed by removing the muscle tissue along the spine, and a partial laminectomy of the T12 vertebrae. Using fine spring scissors, the left dorsal column (DCL; n=2) or a portion of the left dorsolateral funiculus (DLF; n=2) was transected (see Figure A.1). Over six to eight

weeks the ability of the animals to generate a corrective flexion reaction in response to the hole was monitored. A frame-by-frame analysis of the video recordings was used to determine the time of onset of the first visible flexion movement at the hip, knee or ankle following the entrance of the limb into the hole.

All of the animals, when intact, responded to the hole in the characteristic manner described in Chapter 2; following a brief extension into the hole, the foot was quickly flexed from the hole and placed in front of the perturbation, support of the hindquarters was maintained by prolonging the stance phase of the contralateral leg (see Fig. 2.1 in Chapter 2). The latency from the time the foot entered the hole to the beginning of leg flexion measured from the video recordings was in the range of 99 to 165ms (ie. 4-5 video frames after entering the hole). This is very similar to the result from other intact animals documented in Chapter 2.

Lesion of the **dorsal column** in the first animal (refered to as DCL#1) had no noticeable affect on the pattern of undisturbed locomotion or on the corrective flexion reaction. In the week immediately following the lesion, the latency and vigour of the flexion response was identical to that observed while intact.

In the second animal (DCL#2), a completely opposite result was observed. When walking on the treadmill there was a pronounced dragging of the limb at the end of stance phase before a flexion movement occurred. Occasionally the limb would collapse at the onset of stance phase. Over the two weeks following the spinal surgery, the bipedal gait in DCL#2 slowly improved until locomotion appeared relatively normal. Upon encountering the hole the left limb would extend into the hole, and remain in an extended position. A prolongation of stance phase in the contralateral limb was observed as in the intact animal. Once the contralateral limb reached maximum extension behind the animal, the limb would initiate a short flexion movement (ie. a hop). Upon restoration of contralateral support, a weak flexion of the limb in the hole typically occurred. Occasionally, collapse of the hindquarters eventually occurred as the contralateral limb reached maximum extension. The animals hindquarters would then have to be lifted out of the hole. Twenty eight days after the spinal lesion, there was still no corrective flexion response observed when the left limb entered the hole.

It should be noted that in DCL#2, the animal which did not recover a corrective flexion reaction following the spinal lesion, that if the contralateral (right) limb encountered the hole a "normal" corrective flexion response would be initiated. This response was of similar latency to that observed while the animal was intact.

For two weeks following lesion of the left **dorsolateral funiculus** undisturbed overground locomotion (on a smooth tile floor) was slightly impaired in both animals. Occasionally at the onset of stance the limb ipsilateral to the lesion would slide out from under the animal. At the end of stance phase, during bipedal walking on the treadmill, the onset of flexion was not sharply defined in that the dorsum of the paw would drag for a short period before a distinct flexion movement would occur. Two weeks after the lesion, walking on the floor appeared quite normal. The toe drag during treadmill locomotion was also improved.

For the first two weeks neither animal produced a flexion response when the left limb entered the hole. Similar to that described for DCL#2 the limb would be held extended into the hole. The contralateral stance phase was prolonged until the limb was maximally extended, at which point a quick step forward was initiated.

In one animal (referred to as DLF#1) a flexion of the left limb, before the contralateral limb stepped, was first observed 15 days after the spinal lesion. On day 15, 4 out of 8 encounters with the hole generated a flexion response in the left limb. The latency of the flexion response, as determined from the video, was approximately 300 ms (ie. on average flexion of the limb was observed on the 10th video frame after entering the hole). Twenty one days after the spinal lesion, a fairly regular flexion response was observed when the left limb entered the hole. The average latency of the response was 8 video frames or in the range of 248 - 264ms.

The second animal (referred to as DLF#2) did not produce a flexion response in the left limb until day 28. This response could only be generated if the walking was slowed to 0.15 - 0.2 m/s, otherwise the contralateral limb would always step before the limb was removed from the hole. At the slower treadmill speed, the average latency of the flexion response was 248 - 264ms.

In both of the animals with the lesion to the left dorsolateral funiculus, the ability of the right limb to produce a corrective flexion response when encountering the hole was unaffected. The flexion response in the right limb was observed to occur in the range of 3-6 video frames after entering the hole.

The animals were euthanized six to eight weeks after the chronic spinal lesion. A segment of spinal cord around the site of the lesion was removed and fixed in paraformaldehyde. The cord was embedded in wax and serial sections (15 μm) were made through the site of the lesion matter (thanks to N. Hu for preparing the slides). The general extent of each lesion could be discerned by examing the degree to which the white matter was disrupted (see Fig. A1.1). As desired the lesion in DCL#1 was restricted to left dorsal column. The position of the lesion in DCL#2 was also supposed to remove the left dorsal column. However, instead of lesioning the dorsal column, the lesion removed the left dorsolateral funiculus. The lesion in DLF#1 and DLF#2 were placed as desired, removing the left dorsalateral funiculus.

The observation that lesion of the left dorsal column had no noticeable effect on the latency or vigor of the flexion reaction suggests that afferent feedback of a light tactile modality is not an essential ascending signal necessary for generating the normal "Foot-in-Hole" response. However, lesions to the dorso-lateral funiculus ipsilateral to the limb encountering the hole produced a distinct detriment in the production of the corrective flexion reaction. In general, the characteristics of the corrective flexion response three weeks after lesion to the DLF were very similar to that observed in the spinal cat (see Chapter 2). The latency of the flexion response was much slower than normal (>200 ms with the DLF lesion as compared to ~100ms in the intact cat), and the vigor of the flexion was reduced.

The location of the DLF lesion would interupt transmission through a number of ascending and descending spinal pathways. In general, the ascending dorsal spinocerebellar tract (DSCT) and the descending rubrospinal tract (RST) and lateral cortical spinal tract (CST) would be severed. The potential contribution of each of these pathways will be addressed separately.

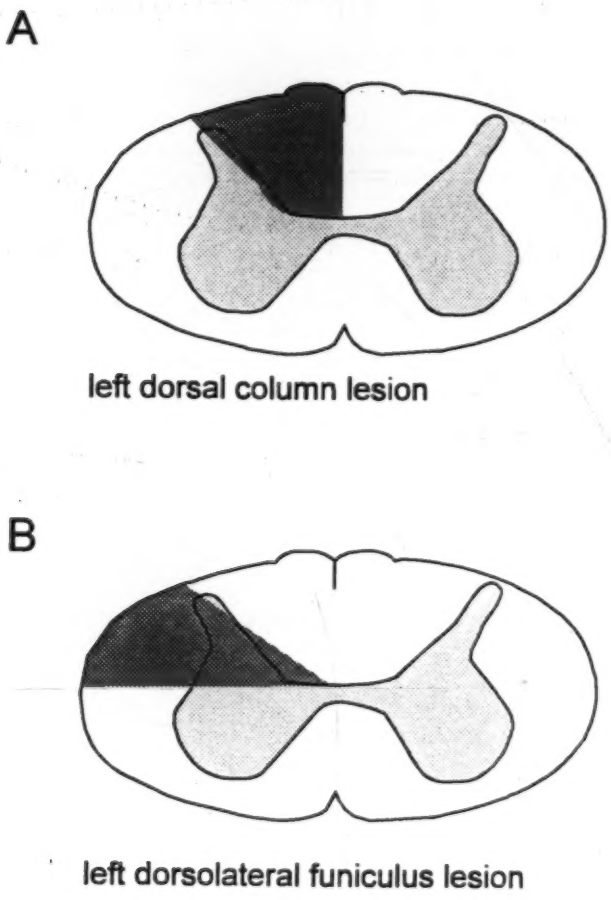

Figure A1.1. Illustration of the regions of the spinal cord removed by selective lesion of the left dorsal column (A) and the left dorso-lateral funiculus (B). The darker shaded regions indicate the relative regions of the spinal cord disrupted by the lesion.

An ascending afferent signal indicating that the step cycle is not proceeding as predicted (that the pattern has been disturbed) is a likely candidate for triggering a descending signal which will compensate for the disturbance. The DSCT transmits information regarding the movement of the limbs. Activity in this pathway has been shown to be largest during early stance phase, possibly a result of the barrage of afferent feedback generated when the limb contacts the ground. The ventral spinocerebellar tract (VSCT) has been proposed to reflect the activity produced by the central pattern generator. It has been postulated that mismatch between the input from the locomotor circuitry in the spinal cord and that indicating the actual state of the limb could serve as a trigger to activate compensatory measures (Armstrong 1986).

The rubrospinal tract is known to facilitate activity in flexor α-motoneurons during swing phase of locomotion. In general stimulation of the red nucleus and the RST increases the amplitude of flexor activity but has little effect on the timing of the locomotor rhythm. It has been postulated that the red nucleus reacts to perturbations in the performance of the central pattern generator, and that any deviations from the normal rhythmic discharge which occurs during walking elicits compensatory movements (Orlovsky 1972, as reviewed by Armstrong 1986). Thus, the absence of activity in the rubrospinal tract could potentially be responsible for the slower and weaker corrective flexion reaction observed after lesion of the DLF.

The potential of the corticospinal tract to alter locomotor activity has already been documented. Drew (1991) found that during locomotion in the cat activity of cells in the motor cortex increase during enhanced flexion of the limb to step over a visually perceived obstacle. Stepping over barriers or walking on ladder rungs is severely impaired in cats with tetrodotoxin inactivation or ablation of the motor cortex (Beloozerova and Sirota 1993). Thus, the removal of this descending drive by lesioning the CST could have been responsible for the loss of the ability to effectively produce a vigourous corrective flexion reaction. Gorassini (1994) documents in her Ph. D. thesis preliminary evidence (n=1) regarding the effect of cortical blockade and subsequent "Foot-in-Hole" responses. Selective blockade of activity in the cortical region associated with the hindlimb, by injecting lidocaine, was shown to have a mixed effect on the

latency of the flexion response. On one day, the corrective flexion response immediately after inducing the cortical blockade was distinctly slower than previous trials. However, the latency of the flexion response during subsequent exposures to the hole occurred within the normal latency. On the second day, no change in the latency of the corrective reaction occurred following cortical blockade. Thus, additional experiments are still required before it can be concluded whether the motor cortex has a specific role in the generation of the flexion response.

References.

Armstrong, D. M. Supraspinal Contributions to the Initiation and Control of Locomotion in the Cat. In: *Progress in Neurobiology*. Vol.26, Great Britain: Pergamon Journals Ltd., 1986, p. 273-361.

Beloozerova, I. N. and Sirota, M. G. The role of the motor cortex in the control of accuracy of locomotor movements in the cat. *J. Physiol.* 461:1-25, 1993.

Drew, T. Visuomotor coordination in locomotion. *Curr. Opin. Neurobiol.* 1:652-657, 1991.

Gorassini, M. A. The control and use of proprioceptive information during purposeful movement. Ph.D. thesis. University of Alberta , 1994.

Orlovsky, G. N. Activity in of rubrospinal neurons during locomotion. *Brain Res.* 46:99-112, 1972.

Appendix II.*

Group I afferent discharge during locomotor activity with the ankle extensors held at a constant length.

Chapter 5 documented that extensor muscle group I afferent feedback is responsible for generating a significant amount of extensor activity during locomotion. One issue left unresolved from that set of experiments was the relative contribution of the muscle spindle group Ia and tendon organ group Ib afferents in the generation of EMG activity. In experiments performed during Chapter 4 of this thesis, it was noted that when the triceps surae were held at longer isometric lengths during locomotor activity that the amplitude of EMG activity and the amount of force generated increased. Taylor et al. (1985) have shown that during isometric muscle contraction in decerebrate walking cats the muscle spindles become unloaded, reducing the group Ia discharge. It is possible that this unloading of muscle spindles may persist when the contraction occurs at a greater muscle length, especially if the amplitude of the contraction is greater. If the extensor muscle spindles should fall silent during locomotor activity then they will not be in a position to contribute in the generation of EMG activity via either monosynaptic (Eccles et al. 1957), disynaptic (McCrea et al. 1995) or oligosynaptic (Guertin et al. 1995) pathways. The work of Jami and colleagues has shown that ensemble tendon organ discharge can reflect the changes in force generated by muscle contraction (see review by Jami 1991). Therefore, an increased tendon organ group Ib afferent discharge during isometric muscle contraction could be responsible for producing the changes in EMG

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* This section of my thesis presents preliminary data from a set of experiments that are still in progress. The fact that these experiments have not been completed is one reason for placing these results as an Appendix. A second reason is that I feel this section is not cohesive with the other Chapters in the body of the thesis which had the corrective response to the absence of ground support as a central theme.

activity. Thus, this Appendix addresses the question as to whether the increased EMG stems from increased spindle activity as the isometric length is increased, or whether the larger contractile forces generate an increased tendon organ discharge which excites the motoneurons through locomotor dependent pathways.

Thirteen adult cats (2.0 - 4.0 kg) were used in this experiment. Each cat was anesthetized with halothane and the trachea was cannulated for continued administration of the anesthetic. One of the carotid arteries was ligated and a cannula to monitor the blood pressure was placed in the other. One jugular vein was cannulated for the administration of fluids and drugs. The following nerves of the left hindlimb were transected: medial gastrocnemius, plantaris, distal tibial, common peroneal, sural, vastus medialis, rectus femoris, sartorius, saphenous, obturator, hamstrings and nerves innervating the tail region. EMG electrodes were sutured into the muscle bellies of SOL, LG, VL and IP of the left hind leg and into IP and LG of the right hind leg. In 5 animals the entire femoral nerve was transected, and therefore VL EMG was not recorded. The calcaneum was detached to free the triceps surae muscle group. In the experiments where VL nerve was left intact, the patellar tendon was transected. A bipolar silicone nerve cuff was placed around the LG/SOL nerve for stimulating the nerve to induce twitch contractions (for use in identifying tendon organ Ib afferents)

The muscle tissue surrounding the L3 - S2 vertebrae was removed to expose the spinal column. A laminectomy of the L5 and L6 vertebrae was performed. To prevent movement of the exposed region during the experiment a rigid piece of wire was cemented with fast drying epoxy and dental acrylic between the L3-4 and the L7 vertebrae. A piece of heavy wire was passed through holes drilled in the iliac crests and cemented into the dental acrylic further prevented movement of the back.

The animal was then moved over a motor driven treadmill, where it was held by a stereotaxic head holder and by clamps which attached to the epoxy on the cat's back. The left hind leg was immobilized above the treadmill surface at the knee and ankle, while the other three limbs were positioned so that they were in contact with the surface of the treadmill. The hip, knee and ankle joints were held at approximately 90° angles. A length

of heavy surgical thread was used to attach the triceps muscle via the calcaneum to a muscle puller equipped with a force sensor (see Figure A2.1).

Small fascicles of dorsal root near the L7-S1 junction were transected and bundles of afferents were teased apart and placed on a hook electrode. Afferents from the LG/SOL muscles were identified as being muscle spindle group Ia or II, or tendon organ group Ib afferents based on their sensitivity to a ramp 4mm stretch, vibration (50μm, 150 Hz), and a twitch contraction of the muscle. The group Ia afferent responds to a ramp stretch with a high frequency discharge, and shows a distinct reduction of activity during the plateau phase. Vibration applied during the plateau often drives the afferent at the vibration frequency (see Fig A2.2). A twitch contraction of the muscle produces a brief silencing of the tonically active spindle primary (see Fig. A2.2B). A tendon organ group Ib afferent is typically identified by its unresponsiveness to a ramp stretch or to vibration of the muscle (not shown). A twitch contraction of the muscle generates a brief burst of group Ib activity corresponding to activation of the muscle fibres and the increase in force (see Fig. A2.1C).

Once 2-4 afferents were successfully isolated and identified the animal was decerebrated and the anesthetic was then discontinued. After decerebration, 5 of the 13 animals began to walk spontaneously on the moving treadmill (speed 0.25 - 0.5 m/s). Rhythmic activity in the fixed left hind leg was coordinated with the stepping movements in the three stepping limbs. If no spontaneous locomotion was observed within 2 hours of decerebration an attempt was made to induce locomotion by electrical stimulation (15 Hz, 0.6 msec duration, 30-100 mA) of the mesencephalic locomotor region (MLR: co-ordinates P2,L4,H6). Stimulation of the MLR was required in 2 animals to produce a locomotor pattern. Five of the animals failed to produce any locomotor rhythm.

The single afferent signal, EMG activity, LG/SOL force and the motor position were recorded. Off-line selected sequences of locomotor activity were digitized and stored on disc using the Axotape data acquisition system (Axon Instruments). The afferent signal was sampled at 2 kHz. The EMG data was first rectified and low pass filtered (100 Hz cut off) before sampling at 700 Hz. Custom written software capable of reading and displaying the Axotape data files was used to calculate the average EMG

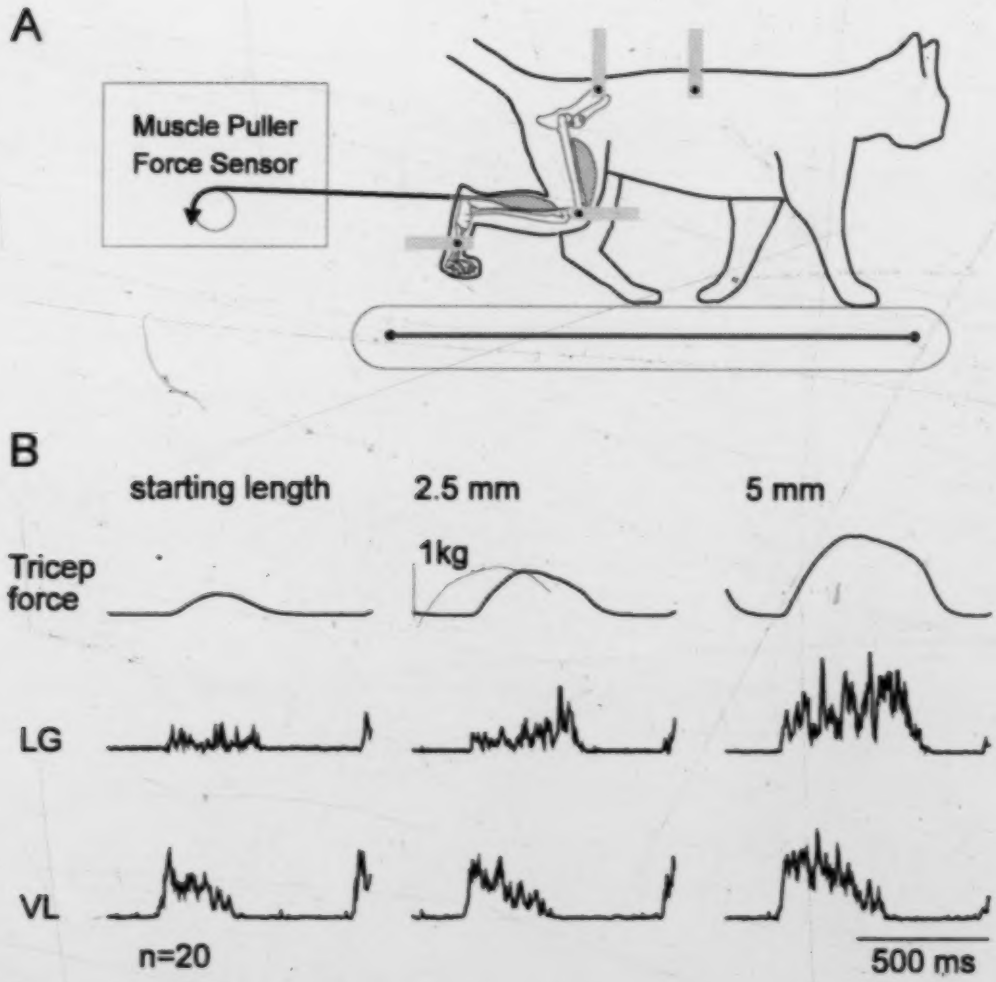

Figure A2.1. Illustration of the experimental setup for holding the triceps surae at various isometric lengths during locomotor activity. A) The iliac crests and the L4 spinous process were held rigid in a stereotaxic mounted to a treadmill. The left knee and ankle joints were also immobilized 90° angles. The LG/SOL muscles were attached to a postition motor. B) At progressively longer the isometric lengths, EMG activity in LG is shown to increase. Activity of the knee extensor VL is also shown to increase activity as the ankle extensors are stretched.

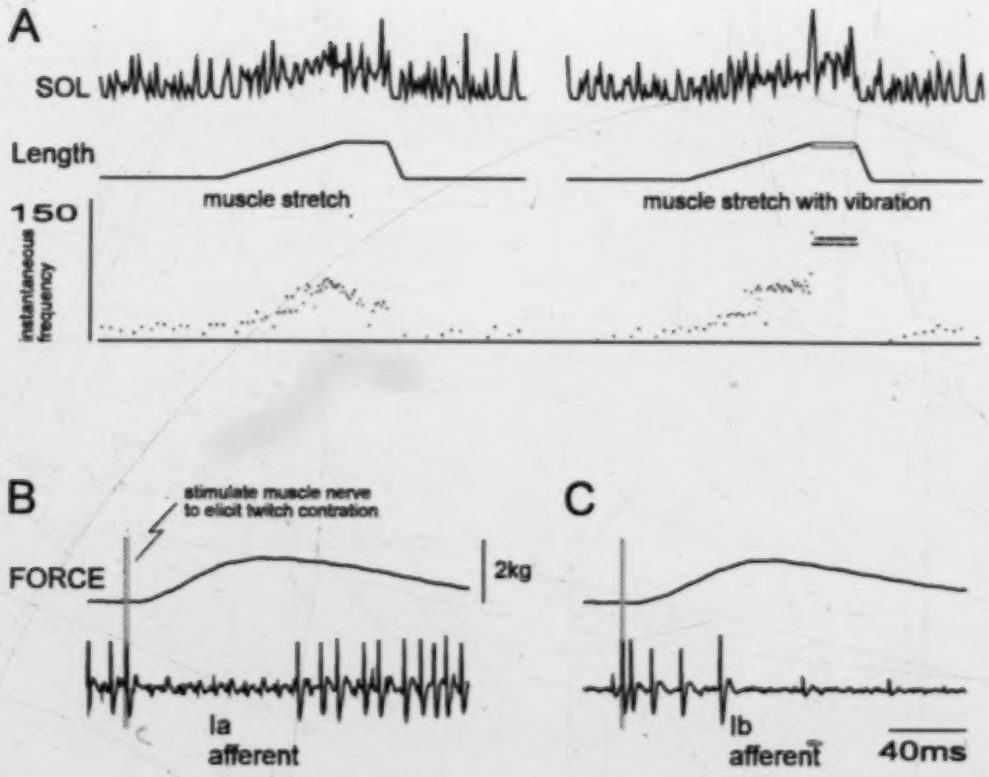

Figure A2.2. Protocol for identifying group Ia or group Ib afferents based on their responsiveness to ramp stretch, vibration, or twitch contraction. A) Group Ia afferent discharge increases with a ramp stretch of the muscle and often show a large dynamic sensitivity (decrease in firing during the plateau phase of the stretch). A distinct characteristic of group Ia afferents is the 1:1 driving produced by vibration (150Hz) of the muscle tendon. B) In response to a twitch contraction of the muscle the group Ia afferents fall silent, whereas the group Ib show a burst of activity with the increase in force.

activity profile for a single step cycle. Custom written soft ware was also used to divide a 1500ms period of activity into 100 bins and calculate the average instantaneous frequency of the afferent for each 15ms bin. Averages were typically generated using the onset of SOL activity, or the onset of the vibration or stretch, as a reference point. Microsoft Excel was then used to calculate the overall average muscle and afferent activity for a 200ms period commencing 100ms after the onset of SOL activity.

EMG response to maintained stretch of triceps surae.

During locomotion the triceps surae muscle was held at variable set isometric lengths (zero, +2-2.5mm and 5mm). For each experiment the "zero" length was set so that there were noticeable changes in the LG/SOL triceps force during the muscle contraction. In response to increasing the isometric length of LG/SOL muscles a distinct increase in the amplitude of EMG activity was observed (see Fig. A2.2). The overall average EMG activity calculated for a 200ms period beginning 100ms after the onset of SOL activity, indicates that SOL activity increased by 150% and LG increased by 240%, as a result of lengthening the triceps surae muscle by 5mm. Furthermore, the increase in activity was not isolated to the triceps surae muscle group in that an increase in VL EMG was observed. In 2 animals, with VL EMG implanted, lengthening the triceps surae by 5mm acted to increase the amplitude of VL activity by 145% and 181%.

Muscle Spindle Afferents. A total of 22 group I afferents from LG/SOL were identified. Eighteen of these afferents, regardless of muscle length, had a pattern of discharge that was very similar to that reported by Taylor et al. (1985). Figure A2.3 illustrates this pattern of discharge for 2 different group Ia afferent. At the onset of activity in the ankle extensors there is a decline in the discharge frequency. The lower level of discharge persisted while the force being generated by the muscle is increasing. Once the muscle force started to decrease, near the end of the extensor burst, the spindle discharge increased and typically reached a peak frequency. If the subsequent flexor burst was of sufficiently long duration then it was typically observed that after the peak in activity the frequency would decrease slightly and hold at a level that was higher than that observed during extensor activity. The pattern would then begin again.

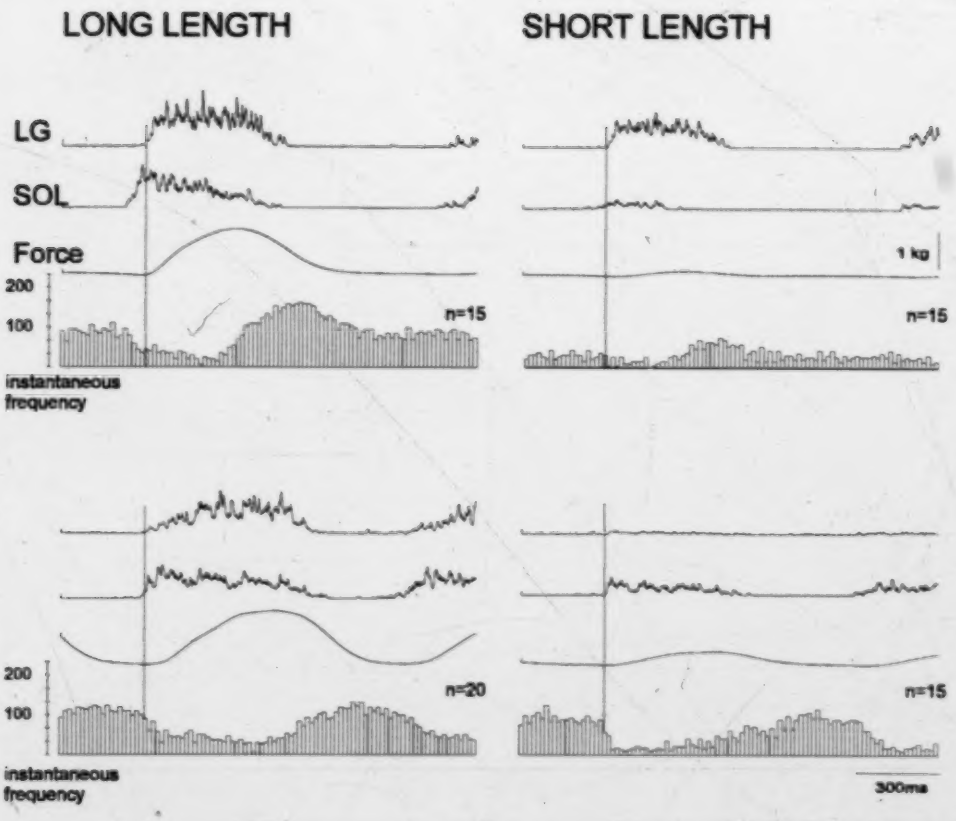

Figure A2.3. The discharge frequency of two representative group Ia afferents during locomotor activity with the LG/SOL muscles held at a "short" or "long isometric length. Similar to Fig. A2.2. extensor activity increases as the length is increased. Note that regardless of the muscle length, the afferent discharge frequency decreases upon the onset of extensor activity. When the force produced by the contraction begins to wane, the instantaneous frequency increases. Even though activity is shown to decrease during the extensor burst, the average level of activity was greater at the longer muscle length. Each bin in the activity profile represents the average instantaneous freqeuncy for a 15ms period.

Four group Ia afferents increased their discharge frequency during the extensor activity and reduced activity between contractions. The onset of activity in these afferents occurred well before the onset of EMG activity.

Tendon organ afferents. A total of 8 tendon organ group Ib afferents were identified. All group Ib afferents showed a similar pattern of discharge. These afferents were silent during flexor activity and began firing upon the onset of extensor activity. The afferent volley continued for the duration of EMG activity and then ceased with the termination of EMG activity.

Average afferent discharge at various isometric muscle lengths.

Nineteen of the 22 group Ia afferents and 6 of the 8 group Ib afferents were recorded from with the muscles held at various isometric lengths during locomotor activity. The average instantaneous frequency was calculated for each afferent in the same manner as that used to calculate the average EMG activity for each isometric muscle length. These averages were then pooled to represent an ensemble group Ia or group Ib discharge. Comparing the average instantaneous frequency produced at the short muscle length to that of the 5mm longer length revealed a distinct increase in afferent activity.

The average ensemble discharge frequency of the group Ia afferents at the short muscle length was 16 Hz, whereas at the longer length the frequency was 29 Hz. This represents an increase of 177%. The tendon organs showed a similar increase in afferent activity; increasing from 40 Hz at the short length to 56 Hz at the longer muscle length, which is a 141% increase.

The present results show that when an extensor muscle is held at a long isometric length during locomotion the amplitude of EMG activity is larger than when the muscle is held at a short length. Taylor et al. (1985) had shown during locomotor activity with the SOL muscle held isometric that the group Ia discharge decreases during extensor activity. Based on this observation it was postulated the level of group Ia discharge would continue to be low even when the muscle was held at a longer length (ie.

unloading of the spindles would persist during contraction), while the level activity of tendon organ activity was predicted to increase. However, this hypothesis did not prove to be true. Both the group Ia and the tendon organ Ib afferent discharge increased as the length of the muscle was increased. Thus, both the muscle spindles and tendon organs could potentially provide the feedback which generates the increase in muscle activity.

References.

Eccles, J. C., Eccles, R. M, and Lundberg, A. The convergence of monosynaptic excitatory afferents on to many different species of alpha motoneurones. *J. Physiol.* 137:22-50, 1957.

Guertin, P., Angel, M. J., Perreault, M. -C., and McCrea, D. A. Ankle extensor group I afferents excite extensors throughout the hindlimb during MLR-evoked fictive locomotion in the cat. *J. Physiol.* 487(1):197-210, 1995.

Jami, L. Golgi tendon organs in mammalian skeletal muscle: Functional properties and central actions. *Physiol. Review* 72:623-666, 1992.

McCrea, D. A., Shefchyk, S. J., Stephens, M. J., and Pearson, K. G. Disynaptic group I excitation of synergistic ankle extensor motoneurones during fictive locomotion. *J. Physiol.* 487(2):527-540, 1995.

Taylor, J. L., Stein, R. B., and Murphy, P. R. Impulse rates and sensitivity to stretch of soleus muscle spindle afferent fibers during locomotion in premammillary cats. *J. Neurophysiol.* 53(2):341-360, 1985.